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Nutritional and Exercise Aspects of Prader-Willi Syndrome and Childhood
Obesity

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14. ABSTRACT

Prader-Willi syndrome (PWS) is the best characterized genetic cause of obesity. To date, two major nutritional phases are identified. Exercise, a valuable tool for the management of obesity, is poorly understood and understudied in PWS. This project studies the nutritional and exercise aspects of PWS and childhood obesity. Preliminary Outcomes: Investigators identified seven distinct nutritional phases which consists of five major phases (phases 0 to 4) with phases 1 and 2 having two sub-phases (phases 1a, 1b, 2a, and 2b). In addition, serum ghrelin levels were significantly elevated in PWS individuals compared to normal sibling controls and individuals with early morbid obesity. Children with and without PWS completed maximal and submaximal aerobic exercise tests on a stationary bike. No major differences appear to exist in the hormonal and metabolic responses to exercise between children with and without PWS except for in 1) growth hormone (PWS do not show an exercise-induced response), and 2) testosterone (PWS demonstrate higher concentrations) Children with PWS respond to exercise with increases in IGF-1 independent of their GH deficiency. No outcomes can yet be reported on resistance exercise. The only outcomes reported at this time in response to resistance exercise are insulin, testosterone and free fatty acids. No major differences appear to exist in the response of these hormones or metabolites between those with PWS and other children.

15. SUBJECT TERMS

Phases, hyperphagia, body composition, exercise, hormones, metabolism

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INTRODUCTION

Obesity is a complex, multi-system disorder whose prevalence is increasing dramatically worldwide, particularly in children. Heterogeneous, complicated, and interconnected factors typically cause obesity. Prader-Willi Syndrome (PWS) is the most frequently diagnosed and best characterized genetic cause of obesity, making PWS a valuable tool in the scientific study of obesity. It is also typically diagnosed before the onset of obesity (Cassidy and Driscoll, 2009). This investigation aims to study the nutritional and exercise aspects of PWS and childhood obesity.

Nutritional Aspects
Classical definitions typically divide PWS into two distinct nutritional stages. We propose to perform a longitudinal study to carefully investigate the following hypotheses: 1) There are 7 (not 2) distinct nutritional phases in PWS characterized by specific abnormalities in metabolism and hormonal levels; 2) Normal weight control, early-onset morbid obese (EMO), and PWS subjects have endocrine and metabolic differences. By comparing individuals with PWS at various ages and in different nutritional stages with obese and lean controls, we will better understand how individuals with PWS progress from an initial failure to thrive to morbid obesity.

Exercise Aspects
In healthy, normal weight youth, the hormonal and metabolic response to exercise strongly contributes to the beneficial adaptations caused by exercise training (e.g., decreased body fat and increased lean mass). Excess body fat appears to alter these hormonal responses. Given the characteristically increased body fat and altered resting endocrine profile of PWS children, this population likely experience atypical hormonal and metabolic response to exercise. As these physiological responses mediate adaptations to exercise training, understanding and documenting these responses will crucially guide the appropriate prescription of exercise to these unique youth. Only a few exercise intervention studies evaluated youth with PWS; none evaluated the hormonal responses to exercise. We therefore propose to conduct an experimental study assessing the hormonal and metabolic responses to an aerobic exercise bout and a resistance exercise bout in normal weight youth, overweight youth, and youth with PWS to ascertain 1) whether the hormonal and metabolic response to exercise differ between youth with and without PWS and 2) if these anticipated differences result from genetic abnormalities or occur secondary to increased body fat.

Exercise Feasibility
The rarity and altered behavior patterns of PWS suggest exercise interventions successful in other youth might fail to suit this unique population. Although planning successful exercise interventions involves prescribing the appropriate physiological stimuli, feasible programs must also minimize parent/caregiver logistical concerns. We therefore propose to survey parents of PWS youth through focus groups and a questionnaire to determine key logistical components that maximize exercise program feasibility. As many barriers challenge parental efforts to ensure their children maintain physically active, we also propose to measure parents' beliefs and attitudes towards physical activity, familial impact on children's physical activity, and other key factors that would assist in sustaining their child's exercise program.

BODY

Nutritional Aspects of Prader-Willi Syndrome and Childhood Obesity (University of Florida Progress Report, February 14, 2013)

Description of procedures

Subjects with PWS have been recruited from various age groups and compared to two age-matched groups: 1) obese individuals who have early-onset morbid obesity (EMO) and 2) a normal weight sibling of PWS control group (Sib.C). All subjects (PWS, EMO and Sib.C) are admitted to the Clinical Research Center (CRC) at the University of Florida for 2 days of intensive study. Testing begins with a thorough history and physical, including a nutritional assessment. Fasting blood work is obtained for a standard chemistry panel, lipid profile, uric acid, insulin and thyroid function tests. A two-hour oral glucose tolerance test is routinely performed. Serum and plasma are obtained in order to measure various appetite regulating hormones and cytokines. Basal metabolic rate and body composition (via DXA) are also assessed. Subjects are followed annually or biennially in the CRC depending upon their age.

We organized the body of this report to address each of the aims of this project.

Aim #1: Establish age range for the various nutritional phases.

Aim #2: Determine if there are particular biochemical and hormonal changes associated with each nutritional phase.

Aim #3: Establish if there are changes in basal metabolic rate (BMR), body fat measurements and caloric input associated with each phase.

Aim #4: Compare the PWS patients to normal weight sibling controls and EMO patients with respect to chemistries, hormonal levels, BMR, body fat and caloric input.

The milestones for this project were extended based on Award W81XWH-09-1-0682.

Aims #1 and #3 have been completed – see manuscript (Miller et al., 2011 – Appendix A). Also, aims #2 to #4 have been completed. Since the last reporting period, we assayed serum ghrelin and plasma leptin levels of individuals with PWS, EMO, and Sib.C between the ages of 0.1 – 21 years. Supplemental funding for this project (under Award W81XWH-09-1-0682) has extended the timeline for this project. A manuscript titled "Hyperghrelinemia in Prader-Willi Syndrome Begins in Early Infancy Long Before the Onset of Hyperphagia" is under review in the Journal of Clinical Endocrinology and Metabolism**.** It is anticipated that a manuscript on the other analytes will be submitted in 2013.

Results

Preliminary findings (as of February 14, 2013)

Aims #1 and #3

Nutritional Phases

We have identified 7 distinct nutritional phases, with 5 major phases and sub-phases of phases 1 and 2 in individuals with PWS (Miller et al., 2011). The initial phase, phase 0, occurs *in utero*, with decreased fetal movements, birth weight and length. In phase 1 the infant is hypotonic and not obese, with sub-phase 1a characterized by difficulty feeding (often requiring feeding via a gastric tube or nasogastric tube) with or

without failure to thrive. This phase is followed by sub-phase 1b when the infant begins to feed better and grows steadily along a growth curve with weight increasing at a normal rate. Phase 2 is associated with weight increase. Sub-phase 2a occurs when the child has an increase in weight without a significant change in appetite or caloric intake, while in sub-phase 2b the child experiences continuing weight increase with an increased interest in food. Phase 3 is characterized by the development of hyperphagia, typically accompanied by food-seeking and lack of satiety. Phase 4 occurs when an individual who was previously in phase 3 no longer has an insatiable appetite and can feel full. This last phase has only been observed in adulthood. It should be noted that not all individuals necessarily go through all the phases and sub-phases delineated above, but most do.

More adult subjects are needed to adequately assess phase 4. With respect to genotype-phenotype correlations we found that the PWS subjects with maternal uniparental disomy of chromosome 15 (UPD) tended to have later completion times for the nutritional phase versus those with paternal 15q11.2 deletions for stages 1b and 2a, but earlier completion times for 2b. However, there were no significant differences between these 2 molecular classes. The change in status from "no growth hormone" to "growth hormone" treatment showed a tendency to accelerate the changes in phases, but this only reached significance for phase 1a ($p<.05$).

A comparison of the adjacent nutritional phases revealed significant differences in fasting IGF-1, glucose, and insulin as well as the BMI z-score, mean resting energy expenditure and percent body fat by DXA (Miller et al., 2011). A significant difference was not found in resting triglycerides and mean respiratory quotient.

Aims #2 and #4

Participant Frequencies and Demographics

We completed participant recruitment under this award in February 2011. Overall, 205 subjects (295 visits) have been admitted to the CRC from the 3 different groups: 1) PWS: 90 subjects; 134 visits; 2) EMO: 15 subjects; 22 visits) and 3) Sib.C: 100 subjects; 141 visits.

Study characteristics for individuals in each group are shown in Table 1. Results for ghrelin and leptin hormonal assays, BMI z-score and body fat percentage (%) through DXA are shown in Table 2.

After adjusting for age, the mean ghrelin level for PWS individuals was 975 pg/ml higher than Sib.C (SE=237, $p<0.001$) and 1147 pg/mL higher than EMO (SE=316, $p<0.001$) individuals. Ghrelin levels decreased on average 76 pg/mL per year of age (SE=15, $p<0.001$) with no statistical evidence of an age by group (PWS vs. EMO vs. Sib.C) interaction ($p=0.16$). In other words, the rate at which ghrelin decreased with age showed no significant difference amongst the three groups. Ghrelin levels also decreased in PWS subjects, albeit non-significantly, on average by 143 pg/mL per unit increase in BMI z-scores (SE=123, $p=0.25$) after adjusting for age.

Table 1. Characteristics of study participants.

	PWS	Sib.C	EMO
0 - 1.99 years			
Subjects	18 (9M, 9F)	12 (7M, 5F)	N/A
Observations	25 (15M, 10F)	14 (8M, 6F)	N/A
Age (years)	1.1 ± 0.5	0.91 ± 0.5	N/A
Mol. Class (Del/UPD/ID)	14/9/2	N/A	N/A
GH treatment (Yes/No)	14/11	N/A	N/A
2 - 4.99 years			
Subjects	41 (22M, 19F)	26 (11M, 15F)	9 (6M, 3F)
Observations	53 (27M, 26F)	28 (11M, 17F)	9 (6M, 3F)
Age (years)	3.7 ± 0.7	3.7 ± 0.9	4.1 ± 0.9
Mol. Class (Del/UPD/ID)	33/18/2	N/A	N/A
GH treatment (Yes/No)	49/4	N/A	N/A
5 - 11.99 years			
Subjects	29 (12M, 17F)	54 (25M, 29F)	20 (10M, 10F)
Observations	41 (17M, 24F)	74 (31M, 43F)	28 (16M, 12F)
Age (years)	7.5 ± 1.8	8.0 ± 1.7	8.2 ± 1.8
Mol. Class (Del/UPD/ID)	26/13/2	N/A	N/A
GH treatment (Yes/No)	36/5	N/A	N/A
12 - 20.99 years			
Subjects	12 (7M, 5F)	23 (14M, 9F)	12 (5M, 7F)
Observations	17 (9M, 8F)	31 (18M, 13F)	15 (7M, 8F)
Age (years)	16.2 ± 2.8	15.5 ± 2.0	15.7 ± 2.7
Mol. Class (Del/UPD/ID)	15/2/0	N/A	N/A
GH treatment (Yes/No)	14/3	N/A	N/A

Table 2: Clinical and hormonal data.

	PWS	Sib.C	EMO	*p1*	*p2*	*p3*
0 – 1.99 years						
Ghrelin (pg/ml)	5521 ± 3696	2883 ± 1172	N/A	0.016* (0.0087**)	N/A	N/A
Leptin (pg/ml)	272 ± 231	216 ± 145	N/A	0.48 (0.39)	N/A	N/A
Weight-for-length (%)	25.07 ± 28.17	57.41 ± 37.50	N/A	0.025* (0.015*)	N/A	N/A
2 – 4.99 years						
Ghrelin (pg/ml)	3113 ± 1898	2556 ± 927	3430 ± 2320	0.12 (0.041*)	0.71 (1.0)	0.30 (0.18)
Leptin (pg/ml)	1389 ± 1785	150 ± 99	2248 ± 1107	<0.001** (0.0040*)	0.098 (0.15)	<0.001** (<0.001**)
BMI *z*-score	0.93 ± 1.55	0.32 ± 1.19	4.29 ± 0.79	0.074 (0.083)	<0.001** (<0.001**)	<0.001** (<0.001**)
Body fat %	24.98 ± 10.47	18.61 ± 6.39	44.04 ± 5.78	0.005** (0.0066)	<0.001** (<0.001**)	<0.001** (<0.001**)
5 – 11.99 years						
Ghrelin (pg/ml)	2476 ± 1332	2111 ± 1013	1645 ± 983	0.21 (0.044*)	0.021* (0.016*)	0.10 (0.55)
Leptin (pg/ml)	2107 ± 1572	397 ± 720	2408 ± 1569	<0.001** (<0.001**)	0.56 (0.25)	<0.001** (<0.001**)
BMI *z*-score	1.62 ± 1.17	0.35 ± 0.92	2.72 ± 0.22	<0.001** (<0.001**)	<0.001** (<0.001**)	<0.001** (<0.001**)
Body fat %	35.08 ± 12.75	20.39 ± 8.05	45.83 ± 4.97	<0.001** (<0.001**)	<0.001** (<0.001**)	<0.001** (<0.001**)
12 – 20.99 Years						
Ghrelin (pg/ml)	2086 ± 885	1233 ± 509	1053 ± 847	0.011* (<0.001**)	0.0085 ** (0.0056**)	0.49 (0.21)
Leptin (pg/ml)	2837 ± 1839	1138 ± 1485	5459 ± 2289	0.0094** (0.0090**)	0.0060** (<0.001**)	<0.001** (<0.001**)
BMI *z*-score	2.10 ± 0.66	0.50 ± 1.08	2.73 ± 0.34	<0.001** (<0.001**)	0.0051** (0.051)	<0.001** (<0.001**)
Body fat %	47.95 ± 8.72	26.00 ± 10.79	54.28 ± 6.09	<0.001** (<0.001**)	0.049* (0.090)	<0.001** (<0.001**)

Note(s): All data are expressed as Mean ± SD. P-values are first given by Mean of Means and then by (Mixed Model).
{ * = *P*-value less than 0.05; ** = *P*-value less than 0.01}
p1 = *P*-value for comparison of PWS vs Sib.C
p2 = *P*-value for comparison of PWS vs EMO
p3 = *P*-value for comparison of Sib.C vs EMO

Children less than 2 years old
PWS children less than 2 years old had significantly higher ghrelin levels than their normal counterparts of same age as analyzed by both the mixed model and mean of means (Table 2; Figure 1). The average weight-for-length percentile of normal control children was significantly higher than that for PWS children, however the body fat % of PWS children as measured by DXA was not significantly different from that of the normal control children (Table 2). There was no significant correlation between ghrelin and weight-for-length or body fat in either PWS or normal children less than 2 years old.

Serum leptin levels did not differ significantly between PWS and normal children below the age of 2 years (Table 2). Leptin levels in PWS children correlated significantly with weight-for-length, but not with body fat % or ghrelin. There were no significant leptin correlations observed in the normal children.

Children 2-4 years old
Serum ghrelin levels in PWS children 2 – 4 years old was significantly elevated relative to normal control siblings (Sib.C) by the mixed model but not by mean of means (Table 2). Ghrelin levels in EMO children did not differ significantly from ghrelin levels in PWS and Sib.C in both statistical models (Table 2).

Serum leptin was significantly elevated in PWS relative to Sib.C. EMO children also had significantly higher leptin levels than Sib.C, however, their leptin level was not significantly different from that in PWS children (Table 2).

Body fat % of PWS children as measured by DXA was significantly more than body fat % of Sib.C children, however, average BMI z-scores did not differ significantly between the two groups (Table 2). EMO children had significantly more body fat % than both PWS and Sib.C children. The average BMI z-score of EMO children was also significantly higher than that of PWS and Sib.C children (Table 2).

Children 5-11 years old
PWS children 5 – 11 years old had significantly elevated ghrelin levels relative to their Sib.C counterparts by the mixed model *(p=0.044)* but not by mean of means *(p=0.21)*. However, their ghrelin level was significantly elevated relative to their EMO counterparts by both statistical models (Table 2). There was no significant difference between ghrelin levels in Sib.C and EMO children (Table 2).

EMO children had significantly higher leptin, body fat % and BMI z-scores than PWS children. Both PWS and EMO children had significantly higher leptin, BMI z-scores and body fat % than their Sib.C counterparts (Table 2).

Teenagers and young adults 12-20 years old
The ghrelin levels of PWS teenagers and young adults 12 – 20 years old was significantly elevated relative to their Sib.C and EMO counterparts (Table 2). There was no significant difference between ghrelin levels in Sib.C and EMO (Table 2).

Plasma leptin, body fat % and BMI z-scores of PWS and EMO subjects were significantly higher relative to their Sib.C counterparts (Table 2). EMO subjects had significantly higher plasma leptin, body fat % and BMI z-scores than PWS subjects (Table 2).

Ghrelin and PWS nutritional phases

Ghrelin (0 - 20.99 years)
a: p=0.2602 (NS)
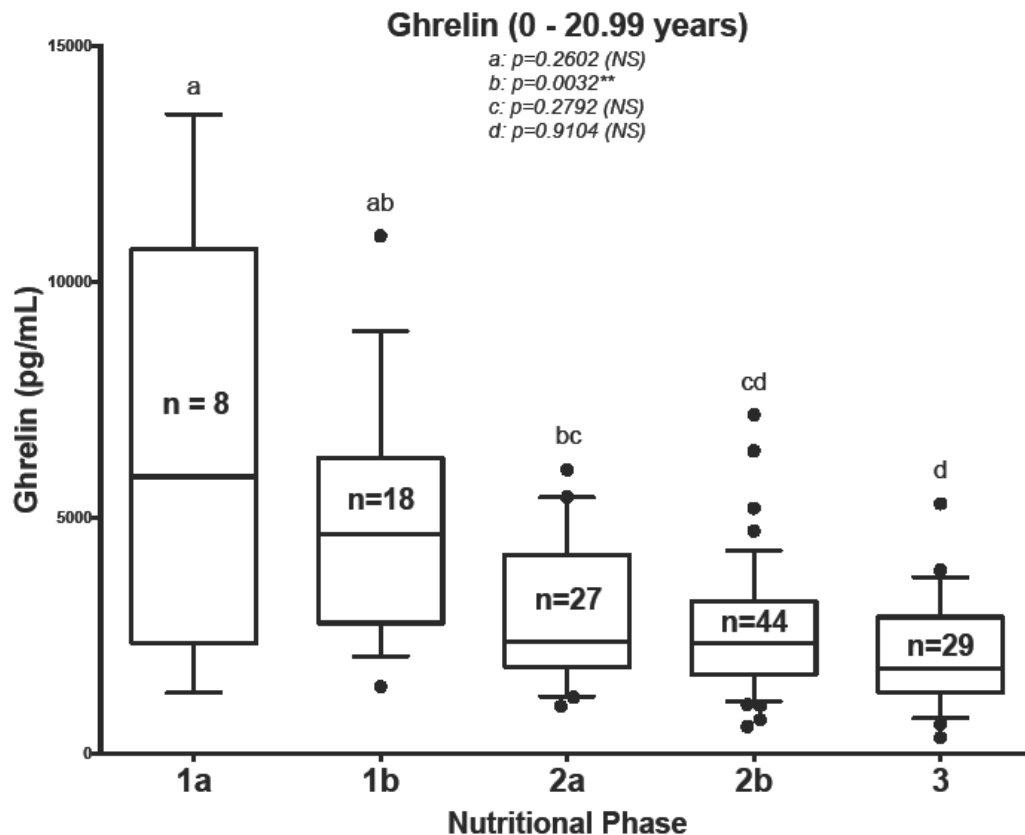b: p=0.0032**
c: p=0.2792 (NS)
d: p=0.9104 (NS)

Figure 1. Ghrelin levels at the various nutritional phases in PWS.

PWS subjects in nutritional phase 1a had the highest ghrelin levels measured (Figure 1). Nutritional phase 1a and 1b together have significantly higher ghrelin levels than phases 2a, 2b, 3, 4, and no further partition of the adjacent phases was significant. We estimate the mean ghrelin levels for combined phases 1a and 1b are 3075 pg/mL higher than the other phases (SE=480, $p<0.001$). After adjusting for nutritional phase, there was no significant association between age and ghrelin levels in PWS subjects. However, nutritional phase is highly prognostic of ghrelin levels in PWS individuals after adjusting for age.

Progression from the early nutritional phases (1a, 1b) to the later phases (2a, 2b, 3) correlated with a significant decrease in ghrelin levels in PWS children between the ages of 0 – 5 years (6043 pg/ml, SE=818 vs 2921 pg/ml, SE=210; $p<0.001$). PWS children 0 – 5 years old in nutritional phase 1a and 1b had significantly higher ghrelin levels than Sib.C children of similar age (6043 pg/ml, SE=818 vs 2656 pg/ml, SE=184; $p<0.001$). However, the ghrelin levels of Sib.C children 0 – 5 years old did not differ significantly from their PWS counterparts in nutritional phase 2a, 2b, and 3 of similar age (2656 pg/ml, SE=184 vs 2921 pg/ml, SE=210; $p=0.3574$).

Ghrelin and growth hormone therapy
We analyzed average ghrelin levels in PWS individuals not on growth hormone therapy relative to PWS individuals on growth hormone therapy. Growth hormone treatment was associated with a mean decrease of 1202 pg/mL (SE=535; $p=0.043$) in PWS ghrelin levels after adjusting for age.

Ghrelin and PWS molecular classes
There were no significant differences in ghrelin levels between PWS patients born with Type 1 and Type 2 deletion. Patients born with UPD (Uniparental Disomy) and ID (imprinting defect) tended to have lower ghrelin levels but it was not significantly different from subjects with deletions.

Discussion
We have demonstrated that serum ghrelin is elevated early on in young PWS children long before the onset of obesity and hyperphagia, confirming the results of a previous study done by the French group (Feigerlova et al, 2008). PWS infants in nutritional phase 1a and 1b had significantly elevated ghrelin levels relative to normal infants. Given that ghrelin levels were the highest in PWS children with poor appetite (Phase 1a), it seems unlikely that elevated ghrelin levels are responsible for the switch to the hyperphagic phases of PWS. However, it has been demonstrated in mice that ghrelin can act to increase fat mass independent of its effect on appetite (Perez-Tilve et al., 2011). It is therefore likely that the elevated ghrelin levels are causing the increased fat mass seen in infants with PWS compared to normal infants with similar body mass indices (BMI). This may explain why PWS infants with lower weight-for-length percentile have similar amounts of body fat with normal infants.

Our results show that nutritional phase is highly prognostic of ghrelin levels in PWS and that progression to the hyperphagic nutritional phases correlates with a decrease in ghrelin levels. PWS children in nutritional phase 2a, 2b and 3 had normal ghrelin levels relative to normal control children of similar age, while PWS children in nutritional phase 1a and 1b had significantly elevated ghrelin levels relative to normal control children of similar age. Given that the age of onset of each nutritional phase varies amongst PWS subjects, analysis of their ghrelin levels by age alone may be erroneous and misleading. Thus it is possible that the inconsistencies in the literature on ghrelin levels in PWS children is a result of reliance on age alone as the major delineating factor.

Our data also suggests that growth hormone therapy may affect ghrelin levels in PWS. Individuals with PWS who were on growth hormone therapy had lower ghrelin levels than those who were off the therapy by as much as 1202 pg/ml. It is possible some of the actions of growth hormone in promoting lean body mass in young PWS subjects lie in its ability to significantly decrease ghrelin levels in early childhood.

Summary of adverse events, unanticipated problems
None.

Summary of deviations/violations
One minor deviation: Basal Metabolic Rate (BMR) not obtained on a subject February 3, 2011 since the BMR cart was not operational.

Summary of complaints
No complaints. The research subjects and their families have been enthusiastic about the study.

Exercise Aspects of Prader-Willi Syndrome and Childhood Obesity (California State University Fullerton and Children's Hospital of Orange County, February 25, 2013)

We organized the body of the report to agree with the technical objectives of this project arm.

Aim #1:   To determine the effects of PWS and body fat on the exercise-induced hormonal and metabolic responses to aerobic and resistance exercise in youth.

Aim #2:   To establish the effects of PWS and body fat on the associations among exercise-induced hormonal and metabolic responses in youth.

Aim #3:   To determine key exercise program factors that may increase program feasibility, accessibility and adherence in youth with PWS.

Project Timeline (February 2008 to February 2013)

Subject recruitment for aerobic exercise arm (Completed):	6 – 34 months
Survey design (Completed):	6 – 12 months
Data collection for aerobic exercise arm (Completed):	7 – 34 months
Focus groups of parents and caregivers (Completed):	12 – 15 months
Survey distribution (Completed):	17 – 22 months
Survey data analysis (Completed):	23 – 28 months
Biochemical/data analyses for aerobic exercise arm:	34 – 50 months
Subject recruitment for resistance exercise arm (Completed):	29 – 49 months
Data collection for resistance exercise arm (Completed):	29 – 49 months
Biochemical/data analysis for resistance exercise arm:	50 – 55 months
Manuscript preparation and submission:	36 – 60 months

We completed data collection on participants in August 2012. We have since completed most hormonal and metabolite assays; unfortunately, several assays required multiple attempts because our very specific population presented either very low or very variable humoral responses. These issues were especially problematic for the catecholamines, forcing us to consult and contract with laboratories at the University of California, Los Angeles to conduct the epinephrine and norepinephrine assays. Finally, we experienced an accidental shut down of our blood storage freezer in July 2012, thawing all unassayed blood samples (endurance arm: catecholamines, extra serum, extra plasma, leptin; resistance arm: all analytes). This unfortunate incident required additional analyses and consultation with biotechnology experts to assess possible sample degradation. Subsequent degradation checks for growth hormone, cortisol, testosterone, and the catecholamines showed no negative effect of thawing. Manufacturer indications support several other analytes also resisted degradation (see Appendix B). Although these procedures required significant and unanticipated time and effort, assessing the samples' stability was crucial to determining the validity of the findings. Given the positive results, we now feel confident moving forward with the remaining assays.

The supplemental contract for this grant also indicated we would analyze blood samples for adiponectin, interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). As newly available evidence demonstrates adiponectin fails to acutely respond to exercise, we will no longer complete this assay. We are currently consulting with colleagues from the University of California, Irvine and McMaster University to finalize procedures for assessing IL-6 and TNF-α in our limited remaining sample volume. We anticipate finishing the hormonal and metabolites assays, data analyses and interpretation <u>by the end of August 31, 2013.</u>

Aerobic Exercise-Preliminary findings

Between February 2012 and February 2013, we analyzed blood samples for catecholamines and insulin-like growth factor binding protein 3. As mentioned above, difficulties with the catecholamine assays led us to current consultations with the Clinical and Translational Laboratory at the University of California, Los Angeles. In addition, our initial insulin-like growth factor binding protein 3 assay yielded unreliable results due to high variability and we will reanalyze these data. The remaining hormone and metabolite measurements will be completed by August 31, 2013.

Results

Data from the aerobic exercise were presented in the progress report dated February 25, 2012.

Adverse Events, SAE's, and Unanticipated Problems

In July 2012, we experienced a malfunction with our blood storage freezer. This event was immediately reported to Melissa Forsythe at the HRPO.

Deviations/Violations

No deviations occurred during this reporting period for the endurance exercise arm of the project.

Complaints

No complaints occurred during this reporting period for endurance exercise arm of the project.

Resistance Exercise Preliminary findings (as of February 25, 2013)

Participant Frequencies

During this reporting period we successfully 1) recruited and tested four youth with PWS, and 2) recruited six and tested five children without PWS (see deviations). We obtained blood from all youth with PWS and four of the five children without PWS. Overall, 52 participants completed the resistance exercise protocol: 12 youth with PWS, 13 normal weight (NW) children without PWS, 17 obese (OB) children without PWS, and 10 adults (ADT) without PWS. We obtained blood samples in forty-eight participants (12 PWS, 12 NW, 14 OB and 10 ADT) and all data below describe only those 48 participants. We used the humoral data from the adult males to demonstrate the efficacy of the protocol in generating a hormonal and metabolic response. This step was required since no previous literature examines children's hormonal and metabolic responses to resistance exercise, necessitating that we first developed an effective protocol. Thus, the adult data were not statistically compared to any experimental group, but instead show the utility of the exercise protocol.

Participant Characteristics

Youth with PWS
Of the 12 participants with PWS, two presented unknown type of diagnosis, while ten presented a deletion. Table 3 presents growth hormone and medication use.

Table 3. Medication use in the 12 youth with PWS, presented as frequencies.

	Current Use	Past Use	Never Used
Growth hormone	7	3	2
Testosterone/estrogen	1	0	11
CoQ10	1	0	11
Diabetes medications	1	0	11
Albuterol	2	2	8
Inhaled steroids	3	0	9
Other medications	2	0	10

Note: A minimum of two consecutive years of growth hormone treatment constituted "current use."

Table 4 displays baseline characteristics. As expected, total body mass, waist circumference, body mass index, body fat percentage and trunk fat percentage of OB and PWS significantly exceeded NW, with no differences between OB and PWS. Additionally, NW children presented significantly lower resting systolic blood pressure (SBP) than OB and PWS.

Table 4. Participant demographics and physiological characteristics presented as frequencies and mean ± SD.

	PWS (n=12)	NW (n=12)	OB (n=14)	ADT (n=10)
Male/Female	6/6	6/6	9/5	10/0
Age (y)	11.8 ± 3.4[*,†]	9.2 ± 1.4	9.6 ± 1.2	23.3 ± 2.4
Height (cm)	146.9 ± 18.2	140.8 ± 10.3	142.8 ± 7.1	177.2 ± 4.8
Body Mass (kg)	55.08 ± 20.64	31.41 ± 6.69[†,‡]	49.04 ± 9.80	77.05 ± 6.36
Waist Circumference (cm)	90.0 ± 13.9	58.1 ± 3.2[†,‡]	81.8 ± 9.1	79.8 ± 4.8
Body Mass Index (kg·m^{-2})	24.75 ± 4.20	15.67 ± 1.31[†,‡]	23.87 ± 3.22	24.54 ± 1.65
Body Fat (%)	43.0 ± 7.7	18.3 ± 4.9[†,‡]	40.3 ± 5.0	12.7 ± 2.9
Trunk Fat (%)	43.1 ± 9.2	17.3 ± 5.3[†,‡]	41.9 ± 5.2	14.8 ± 3.9
Lean Mass (kg)	29.90 ± 12.19	24.29 ± 4.90	27.72 ± 4.96	65.09 ± 7.26
Resting HR (bpm)	79 ± 10	79 ± 11	75 ± 11	58 ± 12
Resting SBP (mm Hg)	109 ± 11	86 ± 10[†,‡]	99 ± 13	107 ± 10
Resting DBP (mm Hg)	64 ± 8	59 ± 8	62 ± 8	66 ± 12

Notes: * = different than NW; † = different than OB; ‡ = different than PWS; $p < 0.05$. One-way ANOVA used. In adults, a BMI <25 kg/m^2 and body fat <25 % indicate normal weight and lean, respectively (ACSM's Guidelines for Exercise Testing and Prescription, 9th Edition).

Results

Exercise Responses
Because we individualized exercise workload to each participant's height and lean body mass (thus equating relative work), PWS stepped onto a lower platform and wore a heavier vest than NW and OB. No significant differences existed among groups in any exercise response (see Table 5).

Table 5. Characteristics of resistance exercise and exercise responses, presented as mean ± SD.

|	PWS (n=12)	NW (n=12)	OB (n=14)	ADT (n=10)
Vest load (kg)	19.91 ± 10.59[*,†]	12.14 ± 2.45	13.88 ± 2.47	32.54 ± 3.63
Step bench height (cm)	23.0[*,†]	28.2 ± 2.1	28.6 ± 1.4	35.4 ± 1.0
Exercise Heart Rate (bpm)	153 ± 19	151 ± 18	164 ± 14	154 ± 13
Peak Heart Rate (bpm)	163 ± 17	160 ± 18	175 ± 17	163 ± 13
End SBP (mm Hg)	133 ± 18	117 ± 14	128 ± 18	156 ± 20
End DBP (mm Hg)	75 ± 14	67 ± 11	63 ± 9	79 ± 11
Test Duration (min)	14.9 ± 4.1	12.6 ± 1.6	12.2 ± 2.0	10.6 ± 0.3

Notes: * = different than NW; † = different than OB; $p < 0.05$.

Hormonal and Metabolic Responses

In this reporting period, we completed analysis of free fatty acids, glucagon, glycerol, growth hormone, insulin, insulin growth-like factor 1, and testosterone. We also contracted the Clinical and Translational Laboratory at the University of California Los Angeles in January to complete catecholamine assays but the laboratory has not provided us with the results yet. We finalized data reduction and therefore include results for growth hormone, insulin testosterone, and free fatty acids.

Results from a three by five repeated measures ANOVA showed a group by time interaction (p=0.004) for growth hormone, suggesting that changes over time differed among the groups. The interaction effect was followed up with two different analyses: 1) separate repeated measures ANOVAs for each group (to ascertain differences in the temporal pattern of the response) and 2) post hocs (to ascertain differences between specific pairwise comparisons). Separate repeated measures ANOVAs for each group showed that NW presented higher GH concentration at all points than 60 minutes into recovery (Min 60) (p<0.001). No significant increase was observed in GH in response to the stress of exercise (p>0.050). OB presented no change in GH in response to exercise or during recovery (p=0.075) likely due to the large variability. PWS showed higher GH concentrations at baseline (Pre-Ex) and immediate post (IP-Ex) than at any time point into recovery. In addition, NW presented higher mean GH concentrations compared to children with obesity (p=0.018) (Figure 2).

Figure 2. Growth hormone response to resistance exercise, presented as means ± SD.
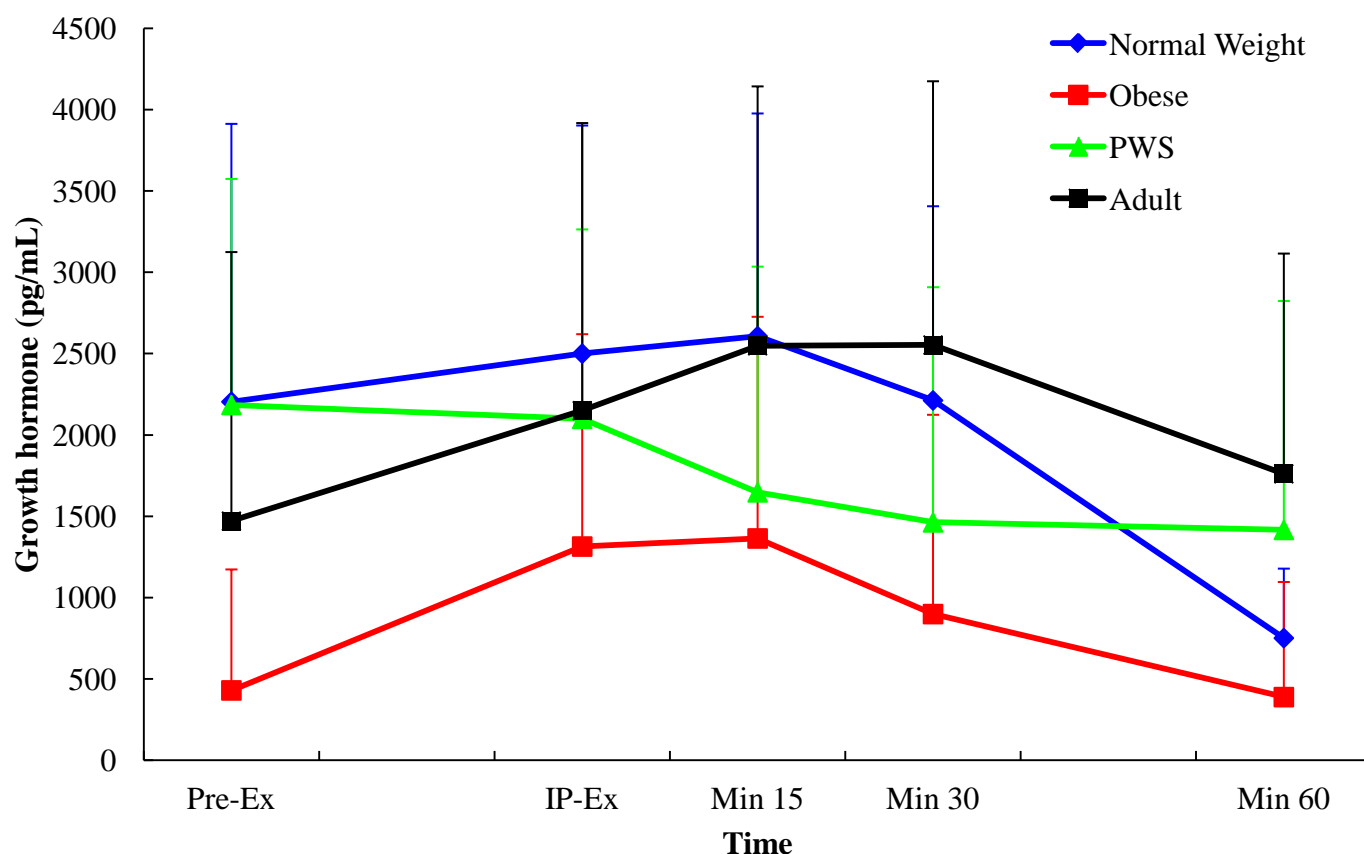
No significant main effect of group or group and time interaction existed for insulin ($p>0.050$ for pairwise comparisons). However, a significant main effect of time indicated that insulin concentrations at Pre-Ex significantly exceeded IP-Ex ($p=0.015$) and Min 60 ($p<0.008$) (Figure 3).

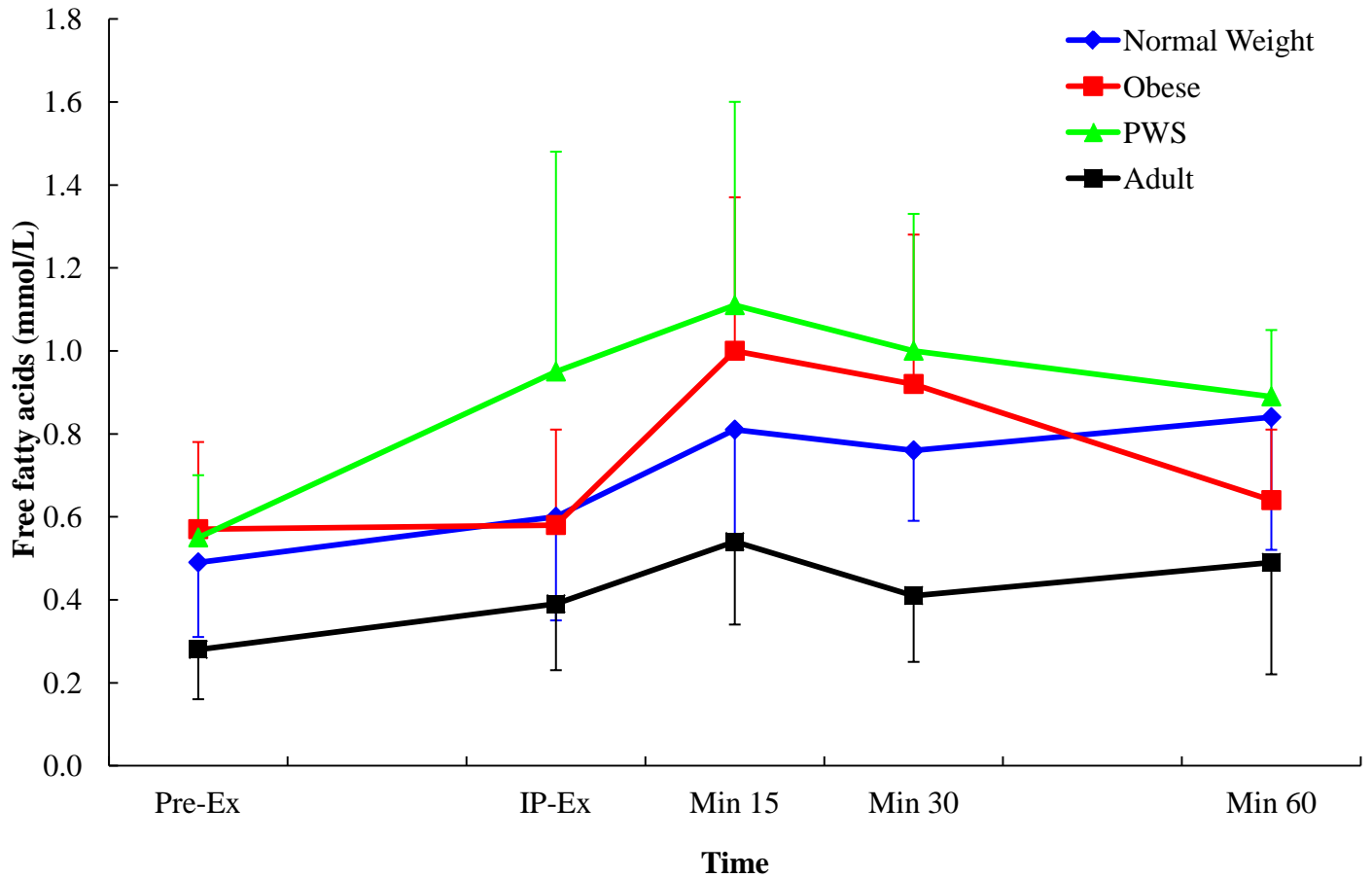Figure 3. Insulin response to resistance exercise, presented as means ± SD.
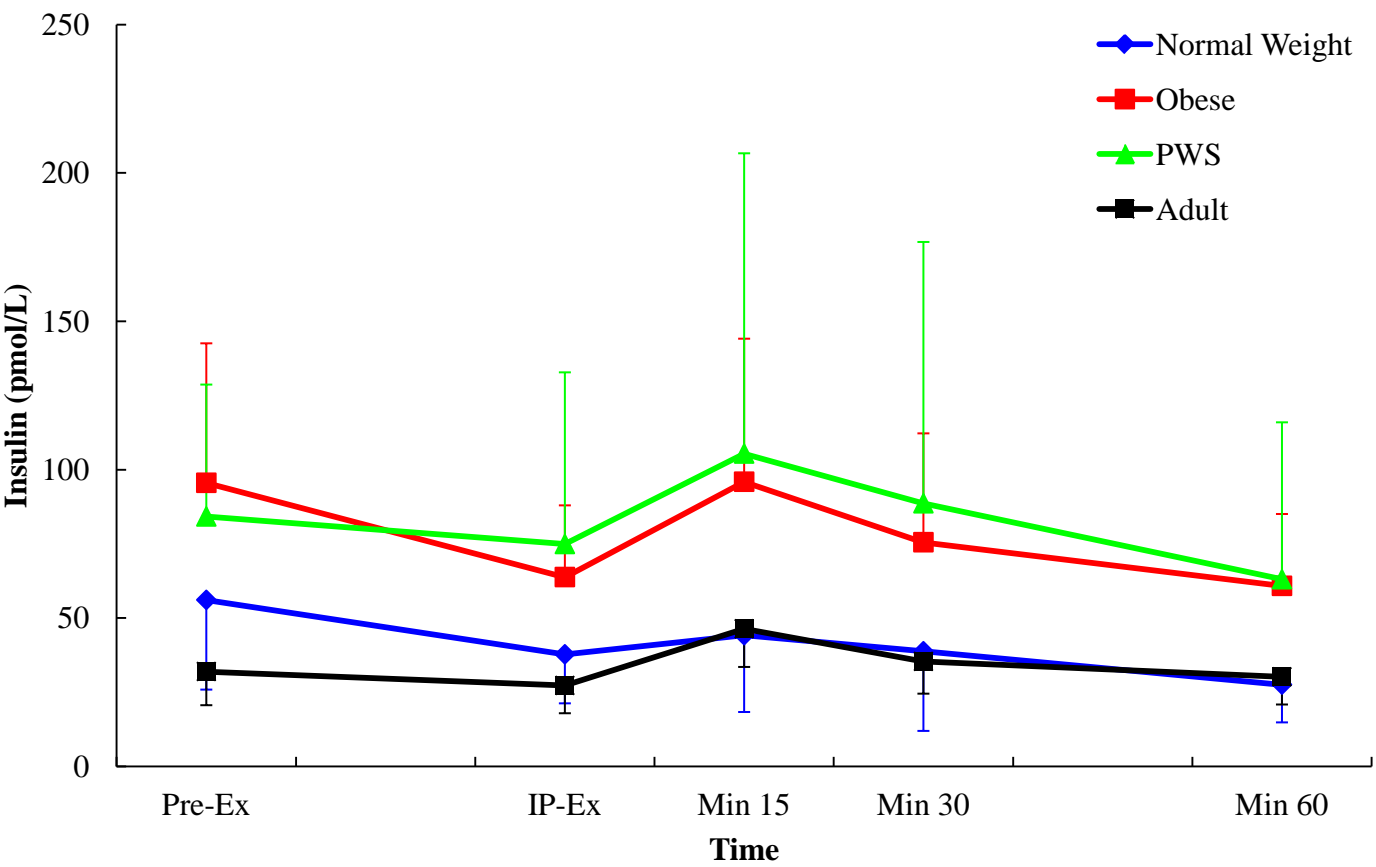
For testosterone, a significantly group and time interaction existed ($p=0.009$). Post hoc comparisons indicated that testosterone concentrations in PWS exceeded NW at all time points except IP-Ex (Pre-Ex: $p=0.043$; 15 minutes into recovery (Min 15): $p=0.025$; 30 minutes into recovery (Min 30): $p=0.044$; and Min 60: $p=0.047$). Although the interaction is significant, no significant changes occurred across time in any group (Figure 4).

Figure 4. Testosterone response to resistance exercise, presented as means ± SD.
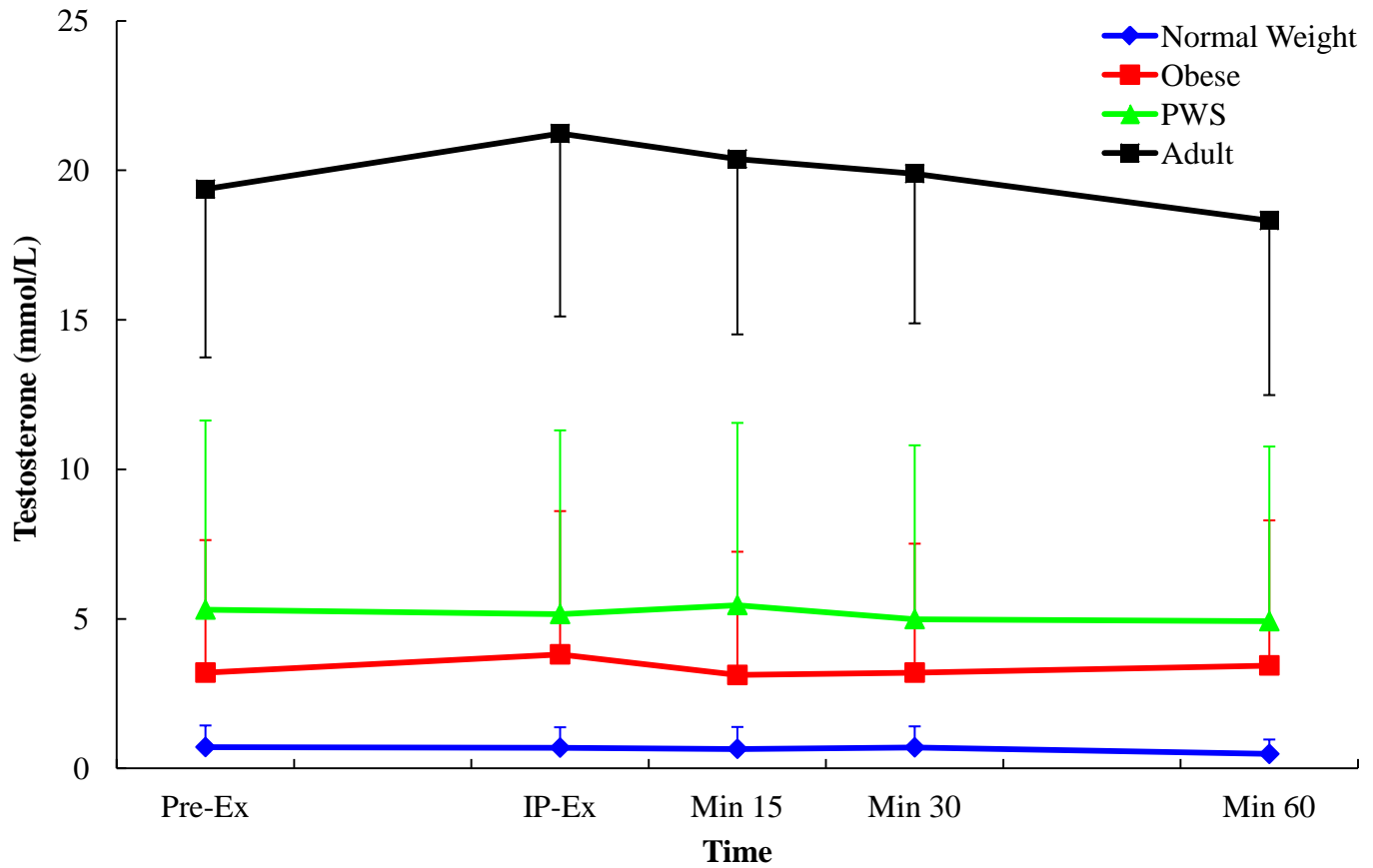
A significant group and time interaction ($p<0.001$) existed for free fatty acids. One way ANOVAs and their corresponding pairwise comparisons showed that selected points after exercise significantly exceeded Pre-Ex free fatty concentration in NW [IP-Ex ($p=0.035$) and Min 15 ($p<0.001$)], in OB [Min 15 and Min 30 ($p=0.001$ for both)], and PWS [IP-Ex to Min 60 ($p<0.007$ for all)] (Figure 5).

Figure 5. Free fatty acid response to resistance exercise, presented as means ± SD.

Discussion

Based on these preliminary data, our resistance exercise results for GH contradict the endurance exercise data, as GH failed to respond to acute resistance exercise. The lack of change in GH in response to exercise in PWS was expected as most people with PWS present hypothalamic pituitary growth hormone deficiency (Cassidy and Driscoll, 2009). In contrast, we expected a significant increase in GH in response to resistance exercise in children of normal weight and those who were overweight. These responses were unanticipated based on our (Rubin, Judelson, Clark in preparation) and others' previous work (Eliakim et al., 2006) and the data displayed very large variability, we will review the individual data points and re-run analyses, if needed.

Similar to studies detailing insulin decreases during and after endurance exercise (Rubin, Judelson, & Clark, in preparation, Eliakim et al., 2006, Hansen et al., 2012 review), our study shows insulin also decreases after short duration resistance exercise. Neither PWS nor obesity appear to affect this decrease which is comparable to the data we presented in response to endurance exercise (Rubin, Judelson, Clark, in preparation).

Although differences at baseline and at most time points during recovery show that those children with PWS have higher testosterone concentrations than those children of normal weight, there seems to be no difference in the response of testosterone to acute resistance exercise among the three groups suggesting that neither obesity nor PWS affected this response. The differences observed between PWS and NW at rest influence all other differences obtained between the groups. But, when the data are evaluated to determine if testosterone concentrations increase in response to exercise, there are no differences among groups as none responded with a significant increase. Testosterone also failed to respond to resistance exercise in adults, indicating that the protocol was perhaps insufficiently intense to trigger testosterone release. There is little, almost none, existing data in adults showing the effect of excess body fat on testosterone release in response to resistance exercise (Hansen et al., 2012). However, data in adults show that those who are obese at rest present lower testosterone concentrations compared to those of normal weight (Trabert et al., 2012). A similar difference at rest between children of normal weight and those overweight were not apparent in our study.

Last, during exercise fat breakdown, which is illustrated by an increase in the concentrations of free fatty acids in the blood, depends on the stimulus received by the sympathetic nervous system. Depending on the protocol chosen and the characteristics of the subjects the increased concentration of free fatty acids may take place immediately during exercise or sometimes during recovery (Powers and Howley, 2012). In this study, exercise stimulated an increase in free fatty acid concentrations in all groups.

We have begun to describe the hormonal and metabolic responses to an acute bout of resistance exercise in children with and without congenital obesity. There are no other studies of this kind. As hypothesized, PWS appears to influence some hormones (such as GH) but not others (in this case insulin). Obesity appeared to not influence any of the hormones presented, but we have several analyses left to complete.

Adverse Events, SAE's, and Unanticipated Problems
Same as endurance study.

Deviations/Violations
Two study deviations occurred this reporting period. The principal investigator withdrew one child participant because the participant's body fat percentage fell within the "at risk for overweight" classification (i.e., between the 85th and 95th percentile), not obese. Additionally, one normal weight participant failed to provide a blood sample due to complications after the catheter insertion.

Complaints
There were no complaints during this reporting period.

KEY RESEARCH ACCOMPLISHMENTS

Nutritional Aspects of Prader-Willi Syndrome and Childhood Obesity: We have been able to identify seven distinct nutritional phases in individuals with PWS. This knowledge should provide a solid foundation for future investigations of the hormonal and metabolic factors associated with these changes. An improved understanding of the various nutritional phases of PWS will not only benefit the treatment and management of individuals with PWS, but also provide valuable insights into the pathophysiology of obesity in general.

We have also shown that hyperghrelinemia begins in early infancy in PWS and decreases as the individual gets older, but is still significantly higher than the sibling control and EMO groups at any age. Therefore, ghrelin is unlikely to be the "key player" in the increased appetite found in individuals with PWS, but is likely responsible for the increased fat mass and decreased lean mass found in PWS.

We are currently exploring whether high ghrelin levels early in life in individuals with PWS correlate with other factors later in life like obesity, appetite drive, behavioral issues and IQ.

Exercise Aspects of Prader-Willi Syndrome and Childhood Obesity: We have characterized the physical activity patterns in PWS, with walking being the most predominant physical activity that individuals with PWS engage in. In addition, we have found that as children with PWS age, they engage in less vigorous physical activity and more moderate intensity activity. These findings are similar to people without PWS. Parents of children with PWS reported that they would be encouraged to enroll their child in a physical activity program if it would improve their children's motor skills and balance as well as stamina and strength. The most common barriers to participating in physical activity were time commitment and travel while less financial constraints and having more time were listed as needs. This information was used to develop a home-based physical activity curriculum which is currently being tested (Active Play at Home).

We have shown that despite the lower stamina and lean mass in children with PWS, they can complete an intermittent aerobic exercise protocol 30 minutes long, as well as a resistance protocol.

We have described most of the acute hormonal and metabolic responses to endurance exercise. Despite baseline differences in IGF-1 and testosterone concentrations and a lack of response of GH to the stress of exercise, youth with PWS respond similarly to acute exercise compared to youth without PWS for several hormones (insulin, glucagon, IGF-1, cortisol, and testosterone). Glycolysis appears not to be a limiting factor during exercise for ATP production, as well as fat breakdown and utilization. Our data suggest that in childhood and adolescence, exercise of cardiovascular nature is encouraged in people with PWS as it triggers a host of endocrine and metabolic responses important for growth and metabolic regulation.

We have begun to describe the hormonal and metabolic responses to resistance exercise. At the moment, we have not identified major differences in the responses for insulin, testosterone or free fatty acids. Once we finish with the analyses of other hormones and metabolites we will have a better picture of the responses to this type of exercise in children with and without PWS.

REPORTABLE OUTCOMES

Publications:

Miller J.L., C.H. Lynn, D.C. Driscoll, et al. (2011). "Nutritional Phases in Prader-Willi Syndrome." *American Journal of Medical Genetics A,* **155**:1040-9. (Appendix A).

Rubin, D.A., Mouttapa, M., Weiss, J.W., Barrera-Ng, A. (2012). Physical Activity in Children with Prader-Willi Syndrome: A Parents' Perspective. California Journal of Health Promotion, Special Issue: Obesity Prevention & Intervention, 10, 57-66 (Appendix C).

Manuscript(s) in review (accepted pending changes):

Rubin, D.A., Cano-Sokoloff, N., Castner, D.M., Judelson, D.A., Wright, P., Duran, A., Haqq, A.M. Update on body composition and bone density in children with Prader-Willi Syndrome. *Hormone Research in Pediatrics,* 2012 (Appendix D).

Manuscript(s) in review:

Castner, D.M., Rubin, D.A., Judelson, D.A., Haqq, A.M. Effects of adiposity and Prader-Willi Syndrome on post-exercise heart rate recovery, *Journal of Obesity, Special Issue: Interdisciplinary Aspects of Childhood Obesity and Physical Fitness*, In Review, 2012 (Appendix E).

Kweh, F.A., Miller, J.L., Sulsona, C.R., Wasserfall, C., Atkinson, M., Shuster, J.J., Goldstone, A.P., Driscoll, D.J. Hyperghrelinemia in Prader-Willi Syndrome Begins in Early Infancy Long Before the Onset of Hyperphagia, *Journal of Clinical Endocrinology and Metabolism,* In Review, 2012.

Manuscript in preparation:

Rubin, D., Castner, D., Ng, J., Clark, S., and Judelson, D. Influence of body fat and Prader-Willi Syndrome on hormonal and metabolic responses to endurance exercise in children.

Presentations:

Driscoll, D.J. (April 2012). Prader-Willi Syndrome: A Model System to Study Imprinting and Obesity. Invited lectured at Diabetes and Endocrinology Research Center, Columbia University School of Medicine, New York, NY (Appendix F).

Wright, P., Rubin, D.A., Mendoza-Castner, D., Judelson, D.A. (June 2012). Evaluation of Body Fat Patterning in Children with Non-Syndromal and Syndromal Pediatric Obesity. Poster session at the American College of Sports Medicine Annual Meeting in San Francisco, CA (Appendix G).

Rubin, D.A. (June 2012). Exercise in Children with Congenital Obesity (Prader-Willi Syndrome) and Non-Congenital Obesity. Special event presentation at American College of Sports Medicine Annual Meeting in San Francisco, CA (Appendix H).

Driscoll, D.J. (October 2012). Prader-Willi Syndrome: A Unique Vehicle into Obesity & Hyperphagia. Special event presentation at the 2nd International Conference on Hyperphagia in Baton Rouge, LA (Appendix I).

CONCLUSION

Nutritional Aspects of Prader-Willi Syndrome and Childhood Obesity: Obesity is now a worldwide problem and has reached "epidemic" proportions. Obesity is the major cause of morbidity and mortality in PWS By comparing analytes from individuals with PWS at various ages and in different nutritional stages with obese and lean controls, we will better understand how individuals with PWS progress from being initially "failure-to-thrive" with a poor appetite to morbidly obese (if untreated) with an insatiable appetite. An improved understanding of the metabolic factors associated with the various nutritional phases of PWS will not only benefit the treatment and management of PWS, but also provide valuable insights into obesity in general. A recent report (*Mission Readiness: Still Too Fat of Fight*) by a group of retired military leaders found that 25% of young Americans cannot join the military because they are overweight and that this is an issue that needs to be dealt with aggressively.

Exercise Aspects of Prader-Willi Syndrome and Childhood Obesity: As in people without PWS, individuals with the syndrome engage in less physically demanding activity as they transition from childhood to adulthood. Therefore, programs that increase physical activity in these individuals are of utmost importance. Children with PWS can complete different exercise protocols of sufficient intensity and duration to generate metabolic and hormonal responses. For the most part, PWS does not interfere with hormones involved in glucose metabolism during exercise (except GH). Similarly, it seems that catabolic processes that take place during exercise, such as glucose and fat breakdown, are not altered in PWS. Exercise is a useful tool to evaluate functioning of the endocrine system under conditions such as PWS or non-syndromic obesity.

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LIST OF APPENDICES

A   Manuscript: Miller J.L., C.H. Lynn, D.C. Driscoll, et al. (2011). "Nutritional Phases in Prader-Willi Syndrome." *American Journal of Medical Genetics A,* **155**:1040-9.

B   Insulin and IGF-1 manufacturer e-mails

C   Manuscript: Rubin, D.A., Mouttapa, M., Weiss, J.W., Barrera-Ng, A. (2012). "Physical Activity in Children with Prader-Willi Syndrome: A Parents' Perspective." *California Journal of Health Promotion, Special Issue: Obesity Prevention & Intervention*, 10, 57-66.

D   Manuscript draft and acceptance e-mail: Rubin, D.A., Cano-Sokoloff, N., Castner, D.M., Judelson, D.A., Wright, P., Duran, A., Haqq, A.M. Update on body composition and bone density in children with Prader-Willi Syndrome. *Hormone Research in Pediatrics,* 2012

E   Abstract: Castner, D.M., Rubin, D.A., Judelson, D.A., Haqq, A.M. Effects of adiposity and Prader-Willi Syndrome on post-exercise heart rate recovery, *Journal of Obesity, Special Issue: Interdisciplinary Aspects of Childhood Obesity and Physical Fitness*, In Review, 2012.

F   Invited Lecture: Diabetes and Endocrinology Research Center, Columbia University School of Medicine, New York, NY, April 2012.

G   Poster: American College of Sports Medicine Annual Meeting in San Francisco, CA, June 2012.

H   Oral Presentation: American College of Sports Medicine Annual Meeting in San Francisco, CA, June 2012.

I   Oral Presentation: 2nd International Conference on Hyperphagia in Baton Rouge, LA, October 2012.

J   CSUF IRB Approval Cover Letter

APPENDIX A

Manuscript: Miller J.L., C.H. Lynn, D.C. Driscoll, et al. (2011). "Nutritional Phases in Prader-Willi Syndrome." *American Journal of Medical Genetics A,* **155**:1040-9.

Nutritional Phases in Prader–Willi Syndrome

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Prader–Willi syndrome (PWS) is a complex neurobehavioral condition which has been classically described as having two nutritional stages: poor feeding, frequently with failure to thrive (FTT) in infancy (Stage 1), followed by hyperphagia leading to obesity in later childhood (Stage 2). We have longitudinally followed the feeding behaviors of individuals with PWS and found a much more gradual and complex progression of the nutritional phases than the traditional two stages described in the literature. Therefore, this study characterizes the growth, metabolic, and laboratory changes associated with the various nutritional phases of PWS in a large cohort of subjects. We have identified a total of seven different nutritional phases, with five main phases and sub-phases in phases 1 and 2. Phase 0 occurs *in utero*, with decreased fetal movements and growth restriction compared to unaffected siblings. In phase 1 the infant is hypotonic and not obese, with sub-phase 1a characterized by difficulty feeding with or without FTT (ages birth—15 months; median age at completion: 9 months). This phase is followed by sub-phase 1b when the infant grows steadily along a growth curve and weight is increasing at a normal rate (median age of onset: 9 months; age quartiles 5–15 months). Phase 2 is associated with weight gain—in sub-phase 2a the weight increases without a significant change in appetite or caloric intake (median age of onset 2.08 years; age quartiles 20–31 months;), while in sub-phase 2b the weight gain is associated with a concomitant increased interest in food (median age of onset: 4.5 years; quartiles 3–5.25 years). Phase 3 is characterized by hyperphagia, typically accompanied by food-seeking and lack of satiety (median age of onset: 8 years; quartiles 5–13 years). Some adults progress to phase 4 which is when an individual who was previously in phase 3 no longer has an insatiable appetite and is able to feel full. Therefore, the progression of the nutritional phases in PWS is much more complex than previously recognized. Awareness of the various phases will aid researchers in unraveling the pathophysiology of each phase and provide a foundation for developing rational therapies. Counseling parents of newly diagnosed infants with PWS as to what to expect with regard to these nutritional phases may help prevent or slow the early-onset of obesity in this syndrome. © 2011 Wiley-Liss, Inc.

Key words: Prader–Willi; nutrition; appetite; weight gain

INTRODUCTION

Prader–Willi syndrome (PWS) is a complex neurobehavioral disorder which is due to the absence of normally active paternally expressed genes from the chromosome 15q11-q13 region. PWS is an imprinted condition with 70–75% of the cases due to a *de novo* deletion in the paternally inherited chromosome 15 11-q13 region, 20–30% from maternal uniparental disomy 15 (UPD), and the remaining 2–5% from either microdeletions or epimutations of the imprinting center (i.e., imprinting defects; IDs) [Bittel and Butler, 2005; Cassidy and Driscoll, 2009]. Clinical features of PWS include hypotonia and poor feeding in infancy which almost always requires some type of assisted feeding for a period of time. Obesity typically begins around age 2 years if the diet is not restricted. Behavioral problems and neuroendocrine abnormalities are also characteristic of PWS [Goldstone, 2004; Davies et al., 2008; Cassidy and Driscoll, 2009].

PWS is classically described as having two distinct nutritional stages: Stage 1, in which the individual exhibits poor feeding and hypotonia, often with failure to thrive (FTT); and Stage 2, which is characterized by "hyperphagia leading to obesity" [Gunay-Aygun et al., 2001; Goldstone, 2004; Butler et al., 2006]. Preoccupation with food, food-foraging, food obsessions and compulsions, and persistent hunger are reported to lead to the obesity that occurs in this syndrome [Gunay-Aygun et al., 2001; Eiholzer et al., 2003; Butler et al., 2006]. The etiology of the switch from poor feeding/FTT to obesity/hyperphagia has yet to be elucidated, but is thought to be associated with abnormalities in the hypothalamic circuitry or peripheral satiety signals [Eiholzer et al., 2003; Goldstone, 2004]. Individuals with PWS have differences in various gut hormones, including high levels of obestatin (an anorexogenic hormone) in infancy, with markedly elevated levels of ghrelin (an orexogenic hormone) in childhood and adulthood. These shifts in gut hormones may possibly correspond to the change between the poor feeding and FTT stage and the hyperphagia and obesity stage of PWS [Eiholzer et al., 2003; Butler et al., 2004; Goldstone, 2004; Bittel et al., 2005; Haqq et al., 2008; Bizzarri et al., 2010]. Individuals with PWS have also been shown to have structural brain abnormalities which may contribute to appetite aberrations [Miller et al., 2007a; Iughetti et al., 2008]. Functional MRI studies indicate that these individuals have an increased reward value to food and have increased activation of the limbic and paralimbic areas of the brain that drive eating behaviors, even post-meal, indicating that brain abnormalities likely also play a role in the appetite in this syndrome [Shapira et al., 2005; Holsen et al., 2006, 2009; Miller et al., 2007b; Dimitropoulos and Schultz, 2008; Hinton et al., 2010].

Animal studies suggest a link between body fatness and appetite, as adipokines produced in adipose tissue play a role in regulating food intake [Stofkova et al., 2009]. When growth hormone (GH) therapy was Food and Drug Administration (FDA) approved for use in individuals with PWS, there was hope that the decrease in fat mass, increase in lean muscle mass, increased metabolic rate, and resting energy expenditure (REE) conferred by GH would result in a decreased appetite in hyperphagic individuals in with PWS [Lee, 2002; Butler et al., 2007]. The effect of GH treatment on the appetite stages in PWS has not yet been reported.

The literature suggests that there is a "switch" between poor feeding and hyperphagia that occurs at approximately 18–36

months of life in individuals with PWS [Eiholzer et al., 2003; Goldstone, 2004; Butler et al., 2006; Haqq et al., 2008; Bizzarri et al., 2010]. However, we have carefully been following the natural history of the feeding behaviors of individuals with PWS for the last 10 years at the University of Florida and for the past 4 years under the auspices of the multicenter Rare Disease Clinical Research Network (RDCRN). We have observed that the changes in appetite and weight gain in PWS are much more gradual and complex than what has been traditionally described. Our group first reported in 2005 our observation that individuals with PWS began to gain excessive weight before the increased appetite develops [McCune and Driscoll, 2005]. We subsequently presented our updated clinical description of the various nutritional phases at the 2006 Second Expert Meeting of the Comprehensive Care of Patients with PWS [Goldstone et al., 2008].

In this study we have investigated our clinical impressions of these more nuanced phases in three different ways. Specifically, we have: (1) carefully characterized and described the nutritional phases of PWS; (2) correlated these phases with objective growth, metabolic, and laboratory data; and (3) examined the effect of GH therapy on the natural history of these nutritional phases.

METHODS
Participants

Families of children and adults with PWS have been enrolled in a natural history study conducted at the University of Florida over the last 10 years. In 2006 this natural history study became part of the Rare Disease Clinical Research Network. Birth measurements were available for 79 individuals with PWS and 84 of their siblings. Complete and accurate growth records and nutritional histories were available on 58 individuals with genetically confirmed PWS, which were used to calculate the onset and duration of the various nutritional phases. In addition we were able to collect laboratory data and concomitantly assign a nutritional phase associated with that data, to 82 individuals with PWS. Many of these individuals had multiple return visits. Fifty-eight percent were male, 90% were white (5% black, 5% Hispanic), and they ranged from 3 months at the time of the first visit to 35 years of age. Thirty-five individuals with PWS had a *de novo* paternal deletion of the chromosomal 15q11-q13 region, 22 had UPD, and 1 had an ID. These individuals came from 16 different states across the United States and three different provinces in Canada. This study was approved by the University of Florida Institutional Review Board, and all adult participants or guardians provided written informed consent and, where appropriate, participants provided assent.

Individuals with PWS were classified into the appropriate genetic molecular classification (i.e., deletion, UPD, or ID) by standard genetic techniques [Cassidy and Driscoll, 2009]. Subjects in the deletion class were further characterized by deletion subtype using the methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay [Bittel et al., 2007; Dikow et al., 2007]. MS-MLPA was done using a commercial MS-MLPA version A1 kit for Prader–Willi/Angelman syndrome (MRC-Holland, Amsterdam, the Netherlands) which contains 25 probes specific for sequence in 15q11-q13. We identified 21% with a Type 1

deletion (i.e., deletion between breakpoints 1 and 3), 34% with a Type 2 deletion (i.e., deleted between breakpoints 2 and 3), and 5% with a unique or atypical deletion.

Metabolic Rate and Body Fat Measurements

REE and respiratory quotient (RQ) were measured on all 82 participants following an overnight fast in the General Clinical Research Center at the University of Florida using a metabolic cart (Parvomedics, Sandy, UT). REE is a calculation of the basal metabolism of an individual, while RQ is a measure of the ratio of the volume of carbon dioxide (V_c) produced by an organism to the volume of oxygen consumed (V_o) [Gropper et al., 2009]. Measurement of RQ provides information about which foods are being used as an energy source. Individuals eating a "standard American diet" have an average RQ of 0.85 indicating that they are utilizing the fat, protein, and carbohydrates they are consuming for energy production. When an individual is being underfed, which promotes use of endogenous fat stores for energy, the RQ is low and is typically closer to 0.7. Overfeeding, however, which results in lipogenesis, increases in the RQ typically to greater than 0.95, indicating that the excess carbohydrates and fats being eaten are being converted into adipose tissue [Gropper et al., 2009]. Only those data points obtained during a steady state (when oxygen consumption and carbon dioxide excretion were stable) were used for data analysis. Body fat was measured using a DEXA (dual energy X-ray absorptiometry; General Electric, Chalfont St. Giles, UK) scanner.

Nutritional Phase Assessment

Nutritional phases were assessed for each individual by two physicians (DJD and JLM) and a dietician (CHL) who have considerable expertise in PWS. Assessments were based on growth charts and nutritional/dietary records, as well as with parental recall. Judgments were made independently and then discussed with the other members of the team. Subjects were excluded if we lacked information to make an adequate assessment of the nutritional phases.

Statistical Analysis

Estimated times (medians and quartiles) to the completion of a nutritional phase (which is reported in Table II as the beginning of the next phase) were assessed by fitting Kaplan–Meier curves. Those individuals who had not completed a phase at last follow-up were censored. Birth parameters (Table III) were compared for subgroups by two-sample t-tests. All two group comparisons were two-sided. For descriptive purposes, $P < 0.05$ was labeled as significant. McNemars test for matched proportions was used to compare in utero fetal movements between subjects with PWS and their sibling controls.

The major analyses contrasted phases 1a, 1b, 2a, 2b, and 3. Sufficient data in phase 4 were lacking for analysis. Because we had repeated measures, both within and between stages, our primary analysis utilized a mixed model approach, with these five phases/sub-phases as fixed categorical independent variables and subjects as random independent variables. We employed a model with a compound symmetric covariance matrix to describe the within-subject associations. There were four analyses where the SAS program Proc Mixed failed to converge, and for those we utilized a fixed repeated measures analysis. These are identified in Table IVb. The following eight dependent variables were utilized: serum IGF-1 measurements, BMI Z-score, glucose, insulin, triglycerides, mean RQ, mean REE, and percentage of body fat by DEXA scan. The analytic strategy was to conduct a five-way analysis first (1a vs. 1b vs. 2a vs. 2b vs. 3) for each variable as a control of studywise error. Whether or not significant at $P < 0.05$, we contrasted the adjacent phases by a similar two-way analysis, but report P-values only if the 5-way analysis was significant at $P < 0.05$. Quantitative estimates for mean differences between adjacent phases are reported in Table IVb as the most important descriptive statistics. For descriptive purposes, we also report means and standard deviations for these phases in Table IVa, but ignore the repeated measures aspects.

RESULTS

We identified seven distinct nutritional phases, with five major phases and sub-phases of phases 1 and 2 in individuals with PWS. The initial phase, phase 0, occurs in utero, with decreased fetal movements, birth weight and length. In phase 1 the infant is hypotonic and not obese, with sub-phase 1a characterized by difficulty feeding (often requiring feeding via a gastric tube or nasogastric tube) with or without FTT. This phase is followed by sub-phase 1b when the infant begins to feed better and grows steadily along a growth curve with weight increasing at a normal rate. Phase 2 is associated with weight increase. Sub-phase 2a occurs when the child has an increase in weight without a significant change in appetite or caloric intake, while in sub-phase 2b the child experiences continuing weight increase with an increased interest in food. Phase 3 is characterized by the development of hyperphagia, typically accompanied by food-seeking and lack of satiety. Phase 4 occurs when an individual who was previously in phase 3 no longer has an insatiable appetite and can feel full. This last phase has only been observed in adulthood. The clinical characteristics of each nutritional phase and sub-phase are delineated in Table I.

Actuarial Ages for Nutritional Phases

While not every single subject experienced every phase, the vast majority of individuals went through each of the phases up to phase 3. Only two of the participants in this study entered phase 4, both during their early 20s. Table II shows estimated actuarial age in years at the onset of each phase. The majority of those who entered phase 3 have remained in this phase during the course of our ongoing natural history study.

Since phase 0 occurs in utero we compared length of gestation and fetal movements, in addition to birth weight, length, and BMI for individuals with PWS versus their unaffected siblings. Fetal movements were decreased in 85% of the newborns with PWS compared to 0% of the siblings ($P < 0.001$) (Table III). Birth weight, length, and BMI were also significantly lower in individuals with PWS versus their siblings (Table III). In addition, mean gestational age for individuals with PWS was significantly different than that of their siblings (38.2 ± 3.0 weeks vs. 39.2 ± 1.6 weeks; $P < 0.001$ by

TABLE I. Clinical Characteristics of the Nutritional Phases

Phase 0   **Decreased fetal movements and lower birth weight**
　　Full-term birth weight and BMI are about 15–20% less than the siblings
　　Typically normal gestational age
　　85% have decreased fetal movements

Phase 1a   **Hypotonia with difficulty feeding (0–9 months)**
　　Weak, uncoordinated suck. Usually cannot breastfeed
　　Needs assistance with feeding either through feeding tubes (nasal/oral gastric tube or gastrostomy tube)
　　or orally with special, widened nipples. Many would die without assisted feeding
　　Oral feeds are very slow
　　Severely decreased appetite. Shows little or no evidence of being hungry
　　Does not cry for food or get excited at feeding time
　　If feeding just occurred when baby "acted hungry" then would have severe "failure-to-thrive"
　　Weak cry

Phase 1b   **No difficulty feeding and growing appropriately on growth curve (9–25 months)**
　　No longer needs assisted feeding
　　Growing steadily along growth curve with normal feeding
　　Normal appetite

Phase 2a   **Weight increasing without an increase in appetite or excessive calories (2.1–4.5 years)**
　　Infant starts crossing growth curve centile lines
　　No increase in appetite
　　Appetite appropriate for age
　　Will become obese if given the recommended daily allowance (RDA) for calories or if eating a "typical" toddler
　　diet of 70% carbohydrates
　　Typically needs to be restricted to 60–80% of RDA to prevent obesity

Phase 2b   **Weight increasing with an increase in appetite (4.5–8 years)**
　　Increased interest in food. Frequently asking "food related" questions
　　Preoccupied with food. Very concerned about the next meal/snack (e.g., "Did you remember to pack my lunch?")
　　Increased appetite
　　Will eat more food than a typical child if allowed
　　Will eat food within their line of sight if unattended
　　Will become obese if allowed to eat what they want
　　Can be fairly easily redirected about food
　　Can feel full
　　Will stop eating voluntarily

Phase 3   **Hyperphagic, rarely feels full (8 years adulthood)**
　　Constantly thinking about food
　　While eating one meal they are already thinking about the next meal
　　Will awaken from sleep early thinking about food
　　Will continue eating if portion size is not limited
　　Rarely (truly) feels full
　　Will steal food or money to pay for food
　　Can eat food from garbage and other unsavory/inedible sources (e.g., dog food, frozen food, crayons, etc.)
　　Typically are not truthful about what they have eaten (i.e. amount and types of food)
　　Will gain considerable amount of weight over a short period of time if not supervised (e.g., some individuals are
　　known to have gained up to 20 pounds in one weekend)
　　Food typically needs to be locked up. Frequently the child will ask the parent to lock the food if the parent has forgotten
　　Will break into neighbors' houses for food
　　Temper tantrums and "meltdowns" frequently related to food
　　Needs to be placed on a diet that is approximately 50–70% of the RDA to maintain a healthy weight

Phase 4   **Appetite is no longer insatiable (adulthood)**
　　Appetite may still be increased or may be normal or less than normal
　　Previously in phase 3, but now a noticeable improvement in their appetite control
　　Can feel full
　　Appetite can fluctuate in this phase, but the key component is noticeable improvement in control of appetite
　　compared to when they were younger
　　Not as preoccupied with food
　　Absence of major temper tantrums and "meltdowns" related to food
　　Onset in adulthood. Could be as early as 20s or as late as 40–50s
　　Most adults have not gone into this phase and maybe some (most?) never will

TABLE II. Estimated Actuarial Ages* at Onset of Nutritional Phase

Nutritional phase	25%-ile	50th%-ile (median)	75th%-ile
1a	Birth	Birth	Birth
1b	0.42	0.75	1.25
2a	1.67	2.08	2.58
2b	3.00	4.50	5.25
3	5.00	8.00	13.00

*Ages given in years.

matched pair t-test). When only full-term pregnancies (gestational age ≥37 weeks) were compared, individuals with PWS still had a significantly lower birth weight than their siblings (3.0 kg vs. 3.5 kg; $P < 0.01$).

Every individual with PWS experienced some difficulty feeding after birth, and thus, were identified as being in phase 1a. Phase 1a lasted until a median age of 9 months (quartiles 5 and 15 months) (Table II). Nine of the 58 individuals we had complete growth records and nutritional data for had severe, prolonged FTT despite receiving what was thought to be adequate calories (i.e., >100 kcal/kg/day) during phase 1a. No associations were found between genetic subtype and prolonged FTT, as seven of these patients had deletion-positive PWS, while two had UPD. There were no significant differences amongst the deletion patients with severe FTT between type 1 and type 2 deletions (three type 1 deletions, four type 2 deletions).

Phase 1b (taking adequate nutrition) lasted to a median age of 25 months (quartiles 20 and 31 months). The end of phase 2a occurred at a median age of 4.5 years (quartiles 3 and 5.25 years). Phase 2b ended (and phase 3 began) at a median age of 8 years (quartiles 5 and 13 years). All but two of the individuals who had entered phase 3 at any age were in this phase when evaluated, with an excessive appetite and lack of satiety.

Deletion Versus UPD

There were no significant differences in length of gestation, birth weight, length, or BMI between infants born with deletion and UPD. Consistent with previous findings, those with UPD had an older maternal age than those with deletion (35.4 years vs. 30.6 years; $P < 0.001$; Table III). There were no differences in the median age of completion of phases between individuals with deletion and those with UPD.

Age at Start of Growth Hormone Therapy

All of the subjects who first enrolled in the study as infants were started on GH therapy. This allowed us to analyze whether starting GH in infancy, as opposed to starting GH later in childhood, made any difference in the tempo or natural history of these nutritional phases. Starting GH in infancy accelerated the pace of phase 1a ($P = 0.039$), thus allowing the infants to enter phase 1b earlier. The age of starting GH did not have any significant effect on the pace or timing of any of the other nutritional phases.

RQ, Body Fat, and Metabolic Changes

Phase 1. Infants in phase 1a who were being fed via nasogastric or gastric tube had a RQ within the normal range from 0.8 to 0.9 (mean 0.89) (Table IVa). However, those infants who were exclusively bottle fed (either with breast milk or formula) had an RQ consistent with underfeeding (0.5–0.7). Percentage body fat was extremely variable amongst infants in this phase but the mean was $22 \pm 9.44\%$ fat (Table IVa and Fig. 1b). Fasting serum insulin levels and insulin-like growth factor levels (IGF-1) ranged from undetectable to the low end of the normal range, while fasting blood glucose levels were normal (Table IVa and Fig. 1c–e). When infants entered phase 1b their percentage body fat did not change significantly, nor did their REE for weight and length, RQ, serum fasting insulin/IGF-1 levels, or blood glucose values (Tables IVa and IVb). BMI Z-scores were not available in phase 1a

TABLE III. Birth Information of Individuals With PWS and Their Siblings (Means and Standard Deviations)

	Type 1 deletion (T1D)	Type 2 deletion (T2D)	Uniparental disomy (UPD)	Siblings	*P* values
Mean gestational age (weeks)	38.1 ± 3.5 (n = 16)	38.1 ± 3.3 (n = 28) All PWS 38.2 ± 3.0	38.1 ± 2.8 (n = 28)	39.2 ± 1.6 (n = 84)	$P = 0.97$ T1D vs. T2D; $P = 0.76$ Del vs. UPD; $P = <0.001$ PWS vs. sibs
Birth weight (kg) [SD]	2.7 ± 0.56 (n = 16)	2.9 ± 0.62 (n = 28)	2.7 ± 0.51 (n = 28)	3.46 ± 0.50 (n = 83)	$P = 0.29$ T1D vs. T2D; $P = 0.40$ Del vs. UPD; $P < 0.001$ PWS vs. sibs
Birth length (cm) [SD]	48.7 ± 3.98 (n = 14)	50.2 ± 3.94 (n = 22)	48.7 ± 3.0 (n = 24)	51.6 ± 3.0 (n = 58)	$P = 0.30$ T1D vs. T2D; $P = 0.29$ Del vs. UPD; $P < 0.001$ PWS vs. sibs
BMI	11.2 ± 1.65 (n = 14)	11.5 ± 1.53 (n = 22)	11.2 ± 1.8 (n = 24)	13.5 ± 2.0 (n = 58)	$P = 0.51$ T1D vs. T2D; $P = 0.66$ Del vs. UPD; $P < 0.001$ PWS vs. sibs
Maternal age at delivery (years)	30.6 ± 5.4		35.4 ± 5.0	31.2 ± 5.4	$P < 0.001$ Del vs. UPD; $P = 0.016$ UPD vs. sibs; $P = 0.13$ PWS vs. sibs

TABLE IVa. Laboratory and Metabolic Parameters of Nutritional Phases of PWS

	1a, N = 11	1b, N = 22	2a, N = 30	2b, N = 54	3, N = 49	4, N = 2
Mean age	0.72 ± 0.4	1.92 ± 0.8	4.46 ± 2.6	7.89 ± 6.3	17.1 ± 9.9	27.9 ± 4.6
(median age)	(0.78)	(1.77)	(3.82)	(5.57)	(15.8)	(26.59)
Weight/length	17%	24%[a] (n = 15)	n/a	n/a	n/a	n/a
BMI Z-score	n/a	−0.7 ± 0.98[a] (n = 7)	0.81 ± 1.37	1.5 ± 1.16	2.1 ± 0.91	1.58 ± 0.84
% Body fat by DEXA	22.0 ± 9.44	19.3 ± 6.8	26.4 ± 13.5	34.0 ± 12.4	45.2 ± 9.9	45.5 ± 10.1
Respiratory quotient	0.89 ± 0.17	0.84 ± 0.13	0.88 ± 0.14	0.89 ± 0.12	0.86 ± 0.12	0.89 ± 0.05
REE	399.9 ± 196.3	675.1 ± 169.7	988.4 ± 312.6	1074.2 ± 367.7	1393.9 ± 431.0	1291.9 ± 174.9
Serum IGF-1 level (ng/ml)	40 ± 25.0	122.7 ± 77.3	211 ± 98.2	279 ± 151.3	291.9 ± 193.8	163.3 ± 23.7
Fasting blood glucose (mg/dl)	72 ± 11.5	77 ± 9.0	80 ± 10.1	83 ± 11.3	88 ± 13.6	83 ± 7.1
Fasting insulin level (mcIU/ml)	1.72 ± 1.9	3.28 ± 2.2	6.36 ± 4.0	10.71 ± 8.4	11.89 ± 12.6	4.39 ± 2.1
Fasting triglycerides (mg/dl)	106 ± 71.0	84.7 ± 39.5	85.8 ± 41.1	91.6 ± 48.0	99.9 ± 51.8	74.2 ± 34.4

n/a, not applicable.

[a]BMI Z-scores from CDC are only available for ≥2 years of age. Some of the subjects in phase 1b were <2 years and some >2 years.

and for many of the individuals in phase 1b due to their young age (i.e., <2 years).

Phase 2. Phase 2a is associated with an increase in body weight without a change in appetite or dietary intake. There were no significant differences in fasting insulin and glucose levels between phase 1b and phase 2a, but fasting insulin levels did trend higher in phase 2a (6.26 mIU/L vs. 3.28 mIU/L; $P = 0.08$) (Fig. 1c,d). As children transitioned between phase 1b and phase 2a they had significant increases in serum IGF-1 levels ($P = 0.002$; Fig. 1e; Table IVb), but no significant change in fasting insulin and blood glucose values. Interestingly, although all of the children were on GH treatment (dose range 0.20–0.26 mg/kg/week) at the time of transition into phase 2a, the IGF-1 levels increased while on a stable dose of GH, suggesting a change in the rate of metabolism of GH. As children transitioned from phase 1b to 2a the REE decreased from 62% (63 kcal/kg/day) of the recommended dietary allowance (RDA) for age (102 kcal/kg/day) to 52% (47 kcal/kg/day with RDA

for age of 90 kcal/kg/day). There was no significant difference in RQ between phase 1b and 2a (0.85 in phase 1b vs. 0.88 in phase 2a; $P = 0.47$).

However, as the average age at which children with PWS enter into phase 2 is associated with a decrease in BMI in typical children, we compared the RQ of the children with PWS entering phase 2 with that of a group of normal control siblings of similar ages. The average RQ of the controls of the same age was 0.76, indicating lipolysis in the typical children as compared to lipogenesis in the children with PWS. Percentage body fat increased from 19.3% in phase 1b to 26.4% in phase 2a ($P = 0.20$) and the BMI SDS increased from −0.70 in phase 1b to 0.8 in phase 2a ($P = 0.032$) (Tables IVa and IVb; Fig. 1a,b).

As individuals transitioned from phase 2a to 2b, which is associated with an increased interest in food, fasting insulin levels continued to increase. (6.36 mIU/L vs. 10.7 mIU/L; $P = 0.01$), but IGF-1 levels and serum glucose levels did not significantly change

TABLE IVb. Comparison of Adjacent Stages by Mixed Models using Compound Symmetric Covariance

Variable	P-value, 5-way	1b−1a, Difference	2a−1b, Difference	2b−2a, Difference	3−2b, Difference
Entries are estimated mean difference (std error) [P-value, two-sided]					
IGF-1	<0.001	130 (42) [0.013*]	92.7 (27.8) [0.0022]	65.9 (39.7) [0.10]	12.6 (41.7) [0.76]
BMI Z-score	<0.001	—	1.31 (0.58) [0.032]	0.80 (0.33) [0.018]	0.66 (0.25) [0.0094]
Glucose	<0.001	6.1 (3.8) [0.18]	3.9 (3.1) [0.22]	2.2 (2.9) [0.45]	4.4 (2.8) [0.11]
Insulin	<0.001	0.72 (1.52) [0.64*]	6.2 (3.4) [0.081*]	4.4 (1.6) [0.010]	1.1 (2.3) [0.64]
Triglycerides	0.72	−21.3 (20.0)	2.5 (12.7)	2.3 (11.8)	9.3 (11.3)
Mean RQ	0.85	−0.052 (0.062)	0.033 (0.046)	0.009 (0.033)	−0.017 (0.025)
Mean REE	<0.001	277 (73) [0.0011]	297 (92) [0.0032]	98 (107) [0.36]	373 (109) [0.0012]
DEXA fat (%)	<0.001	5.5 (3.2) [0.12*]	4.7 (3.6) [0.20]	7.2 (3.7) [0.054]	13.2 (3.1) [<0.001]

The four P-values with * were actually done by fixed effects, repeated measures, as the mixed model failed to converge.
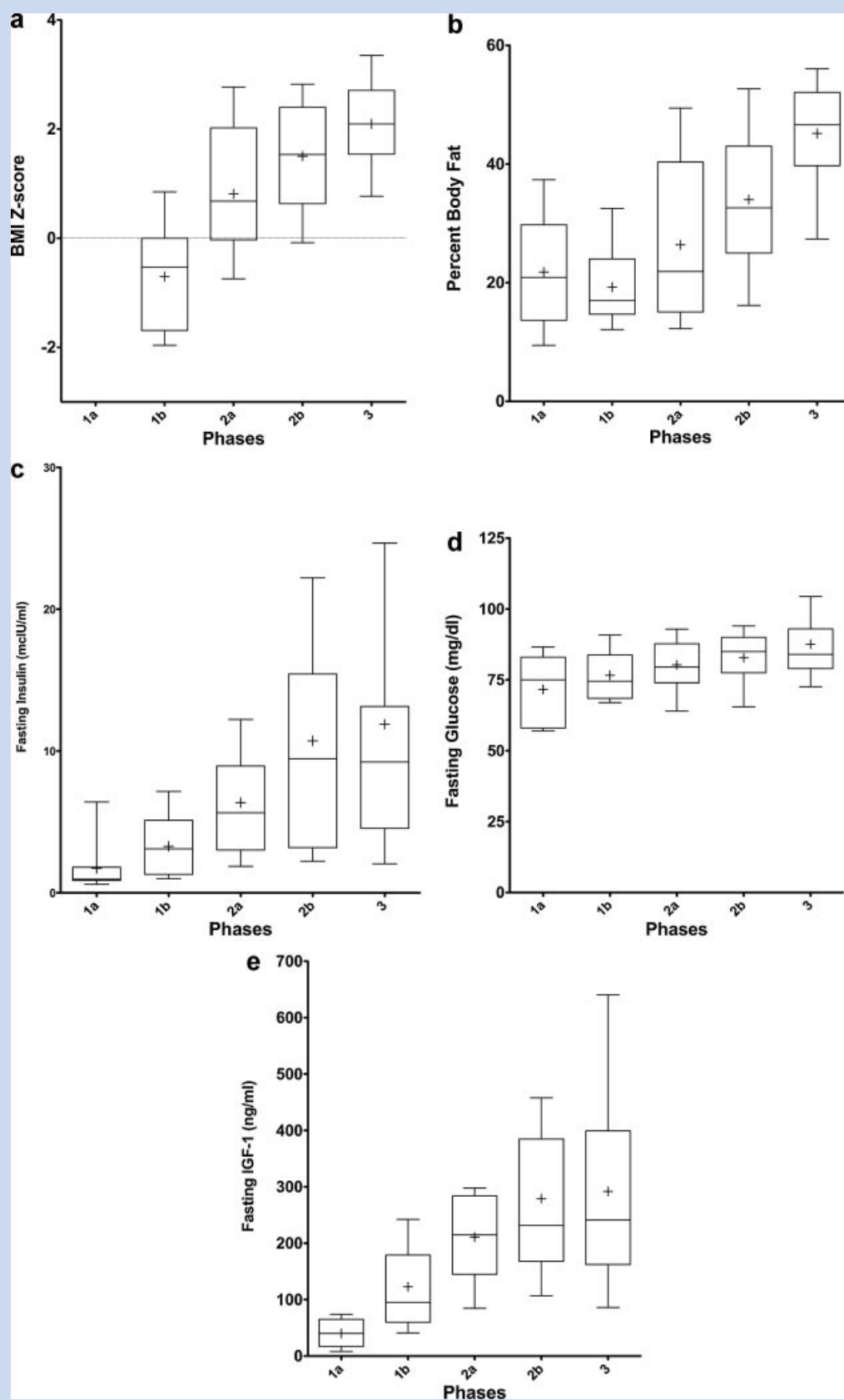
FIG. 1. All figures contain information presented as boxplots. The bottom of the box indicates the 25th centile, the line within the box indicates the median, the cross within the box indicates the mean, and the top of the box indicates the 75th centile. The whiskers above and below the box indicate the 90th and 10th centiles. a: BMI Z-score by phase. (For individuals in phases 1b-3; z-score not available for phase 1a as BMI Z-scores from CDC are only available for ≥2 years of age.) b: Percentage body fat by DEXA by phase. c: Fasting insulin levels by phase. d: Fasting blood glucose levels by phase. e: Serum IGF-1 levels by phase.

(Tables IVa and IVb). BMI SDS increased from 0.8 in phase 2a to 1.5 ($P = 0.018$) which was due to an increase in percent body fat from 26.4% to 34.0% in phase 2b ($P = 0.05$; Table IVb and Fig. 1a,b). RQ remained stable during this transition (0.88 vs. 0.89; $P = 0.78$), while REE decreased to 31 kcal/kg/day which is 44% of the RDA for age (70 kcal/kg/day).

Phase 3. Individuals in phase 3 have an increased appetite with decreased satiety, but they had no significant changes in their fasting insulin, IGF-1, or blood glucose values as compared to individuals in phase 2b. However, BMI SDS increased to 2.10 ($P = 0.0094$ vs. phase 2b) and percent body fat increased to 45.2% ($P < 0.001$ vs. phase 2b) (Fig. 1a,b). RQ remained stable in this phase.

Phase 4. Only two adults in this study had transitioned to phase 4. Additional research is needed with more adults to identify changes in RQ, hormonal levels, or body fat associated with this phase.

DISCUSSION

In contrast to the long-held view that people with PWS go through just two nutritional phases, this study found compelling evidence for five major nutritional phases. Data also point to sub-phases within the first two phases, which further highlights the complexities of the nutritional phases and transitions in individuals with PWS.

Although in the literature, phase 1 begins in infancy with poor feeding and FTT, abnormalities in nutrition in PWS actually begin *in utero.* Here, we propose a phase 0 to reflect these abnormalities and to call attention to the importance of the prenatal environment in subsequent development. In our study the mean birth weights and BMIs of PWS probands was about 15% and 20% less, respectively, than their siblings. Similar reduced birth weights in infants with PWS have also been reported by our group and others [Butler et al., 2009, 2010].

There were 9 of 58 individuals who had severe FTT despite adequate caloric intake during phase 1a. We hypothesize that these individuals had a higher metabolic rate than their peers who did not have difficulty gaining weight. Support for this hypothesis comes from the PWS mouse model with a deletion of the snoRNA *Snord116* gene [Ding et al., 2008]. These mice have an increased appetite and caloric intake, but remain lean due to their increased metabolic rates compared to their wild-type littermates [Ding et al., 2008]. Unfortunately, most of the individuals in our study with severe FTT did not have their metabolic rate measured until well after their FTT had resolved. Alternatively, the FTT in these individuals could be due to decreased absorption of nutrients. This subset of individuals will need to be prospectively studied in the future. Future studies need to identify the metabolic rates and nutrient absorption in this high-risk subset of infants, and how, or if, their longer periods of FTT impact their subsequent development.

Interestingly, we found that phase 1b ended at a median age of 2.1 years, which is often cited as the beginning of Stage 2 (i.e., increased appetite and obesity) in the traditional nomenclature [Eiholzer et al., 2003; Haqq et al., 2008; Bizzarri et al., 2010]. However, we found that when individuals enter phase 2a they began to gain weight without any change in appetite or calories [McCune and Driscoll, 2005; Goldstone et al., 2008]. This observation has also recently been independently confirmed by researchers in the United Kingdom [Butler et al., 2010]. The age of onset of increased interest in food (i.e., phase 2b) in our study was not until a median of 4.5 years. However, the onset of the classically described "insatiable appetite" phase did not begin until a median age of 8 years, which is much older than what has traditionally been thought.

Because PWS is now typically diagnosed in infancy we are better positioned to offer parents prospective advice on these nutritional phases. While we do not yet know what triggers transitions between phases, we hypothesize that there is likely a decrease in metabolic rate and/or an increase in the absorption of calories and nutrients from the diet as children enter phase 2a, which then worsens in subsequent nutritional phases. In these children the REE decreased from approximately 60% of the RDA for age in phases 1a and 1b to 52% of the RDA in phase 2a. The REE then continued to decrease compared to the RDA for age as the children progressed through the nutritional phases. Based on these data, we recommend that parents have their children's length and weight measured monthly. When increasing weight gain without a change in calories is noted, we typically need to recommend that the parents decrease the caloric intake to about 50–80% of the RDA for age as we continue to follow the growth parameters closely for each individual. In so doing, it is important to ensure that the diet remains well balanced with 30% fat, 45% carbohydrates, and 25% protein. If children with PWS remained on a typical American toddler diet which can be composed of 60–70% carbohydrates, their obesity would be even worse as their increased RQ compared to typically developing toddlers suggests that they are prone to convert extra carbohydrates into adipose tissue.

Although parental counseling and caloric restriction have not changed the tempo or timing of the phases, we have been able to achieve great success with many of our infants and young children in keeping the weight for height normal before the child enters phase 2b. When we retrospectively reviewed growth charts of our older individuals with PWS who were typically not diagnosed until 8–12 years of age, we found that they were already obese when they entered phase 2b, so the increased interest in food served to worsen their existing obesity. Parents of our patients diagnosed in infancy thus have the opportunity to institute food-related modifications and healthy eating habits well before the child's appetite or interest in food increases. As a result, when phase 3 begins it is often less severe in those families who have implemented early intervention measures versus what has been traditionally described in the literature.

Best practice in early intervention in PWS also now includes recommendations for GH therapy. GH therapy decreases fat mass and increases muscle mass. Preliminary data also suggest that it may have a beneficial effect on weight gain, and possibly appetite, in individuals with PWS [Myers et al., 2000; Burman et al., 2001]. The present study found that GH therapy in infancy significantly shortened phase 1a, allowing infants to spend more time in phase 1b, during which time they gain weight appropriately. Although at this point GH therapy did not significantly affect any of the other nutritional phases, the majority of participants who started GH treatment in early infancy are not yet old enough to have progressed

through phases 2b, 3, or 4. Follow-up data on these children are needed before drawing conclusions about the efficacy of infantile GH therapy on the progression or timing of the later nutritional phases.

Although this study identified novel ways of conceptualizing nutritional phases in PWS, it also had certain limitations. First, some of the data on older individuals is retrospective and based on analysis of growth charts and parents' memory. However, we have excellent historical data on a number of our older patients (many of whom have been followed by our group for 10–20 years and who were diagnosed in early infancy) which documents the progression of these individuals through the various nutritional phases which we have described. Further prospective work is clearly needed on the life course of the nutritional phases. A second weakness is that the study did not include measurements of appetite-regulating hormones and neurotransmitters as participants progressed through the various stages. Even so, this study provides a critical step in describing and verifying these various nutritional phases and setting the stage for future collaborative rare disease consortium studies on shifts in hormones and neurotransmitters as individuals transition through various nutritional phases. Data are especially needed on transitions between phase 3 and 4, and mechanisms that explain why some adults have a lessening of their hyperphagia while others do not. Although there were only two individuals in this study who had entered phase 4, we have seen several adults in clinic who have entered this phase, but we do not have research measurements on them at this time.

In summary, we have been able to identify seven distinct nutritional phases in individuals with PWS. This knowledge should provide a solid foundation for future investigations of the hormonal and metabolic factors associated with these changes. An improved understanding of the various nutritional phases of PWS will not only benefit the treatment and management of individuals with PWS, but also provide valuable insights into the pathophysiology of obesity in general.

ACKNOWLEDGMENTS

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APPENDIX B

Manufacturer e-mails for insulin and IGF-1 assays

E-mail regarding insulin assay

From:	Todd Hendrich <todd.hendrich@merckgroup.com>
Sent:	Friday, August 03, 2012 8:20 AM
To:	drubin@fullerton.edu
Subject:	Human Insulin Question [ref:_00D30akC._50080Kh4FS:ref]

Daniela,

We did not do freeze/thaw studies with sample and this kit but we did do this with our human metabolic hormone panel which measures insulin. We saw little to no degradation of insulin after 3 freeze/thaw cycles and the recommendation from that kit developer was to not do more than 2 freeze/thaw cycles with samples. So as far as the freeze/thaw cycle you should be ok. We also tested leaving the samples at 4C for a day and insulin was very stable. I know this is not exactly the information you need, but hopefully this helps. Let me know if you have any more questions.

Todd Hendrich
Technical Applications Scientist
EMD Millipore

6 Research Park Dr.
St. Charles, MO 63304
636-441-8400
Todd_Hendrich@merckgroup.com
ref:_00D30akC._50080Kh4FS:ref

E-mail regarding IGF-1

From: Liliana Greenwald <lgreenwald@alpco.com>
Date: Monday, August 6, 2012 6:38 AM
To: "'drubin@fullerton.edu'" <'drubin@fullerton.edu'>
Subject: FW: Technical Question

Dr Rubin,
Please find the information requested below. I hope this is helpful.

11CORHU-E01: Analyte stable at 4°C for up to 24 hours
11TESHU-E01: Analyte stable at 4°C for up to 24 hours
22IGFHU-E01: IGF-I levels are usually not affected by improper
Handling or storage. They remain stable over several days in normal and in various clinical situations even under conditions of high temperature 98.6°F (37°C).
17 BCTHU-E02 RES: Analyte stable up to 6 hours at 2 – 8 °C.
17TCTHU-E03RES: Analyte stable up to 6 hours at 2 – 8 °C.

Kind regards,

Liliana Greenwald. MS.
Product Support Specialist
ALPCO Diagnostics
(800)592-5726 ext 258
www.alpco.com

APPENDIX C

Manuscript: Rubin, D.A., Mouttapa, M., Weiss, J.W., Barrera-Ng, A. (2012). Physical Activity in Children with Prader-Willi Syndrome: A Parents' Perspective. California Journal of Health Promotion, Special Issue: Obesity Prevention & Intervention, 10, 57-66.

Physical Activity in Children with Prader-Willi Syndrome: A Parents' Perspective

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[1]*California University Fullerton, Fullerton*
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Abstract

Background. Physical activity (PA) is vital for the management of weight among those with Prader Willi Syndrome (PWS). However, little is known about characteristics of PA in individuals with PWS.
Objective/Hypothesis. To assess from the parent's perspective, PA levels among individuals with PWS, their preferences for specific activities, and perceived benefits, barriers, and resources needed to participate in PA.
Methods. Participants were 90 parents and caregivers of a child with PWS, predominantly in California. Survey questions included their child's participation in different PAs, perceived benefits and barriers to enrolling their child in a PA program, and perceived needs to facilitate their child being physically active.
Results. Walking was the predominant PA, representing 66% of the activity time across all age groups. Children 10-17 years old engaged in more moderate intensity PA per week than children 5-10 years old. Children 5-10 years old engaged in more vigorous PA than those 18+ years old.
Parents reported that they would be encouraged to enroll their child in a PA program if it would improve their children's motor skills and balance (78.2%) as well as stamina and strength (74.4%). Time commitment and travel were most common barriers. Less financial constraints (70.1%) and having more time (54.7%) were listed as needs.
Conclusions. Similar to individuals without PWS, vigorous PA declines with age. It is recommended that caregivers and health care providers emphasize the role of vigorous and bone-strengthening PA as children with PWS approach adolescence.

Keywords:

Introduction

Prader-Willi syndrome (PWS) is the most characterized form of congenital obesity and results from an alteration of the paternal chromosome 15. The prevalence of PWS ranges from 1 in 10,000 to 1 in 25,000 live-births (Butler, Phillip, & Whitman, 2006). The syndrome is characterized by infantile hypotonia with failure to thrive at birth. During childhood the onset of hyperphagia takes place and strict dietary management is needed. Other characteristics of the syndrome include growth hormone deficiency (Burman, Ritzen, & Lindgren, 2001), poor motor coordination and muscle tone, increased adiposity, and low energy levels (Butler, Phillip, & Whitman, 2006). Individuals with PWS also present a lower proportion of muscle mass compared to people of the same body size, therefore, their energy expenditure is considerably lower when compared to controls (Butler, Theodoro, Bittel, & Donnelly, 2007). In addition, individuals with PWS present behavioral challenges and wide ranges of cognitive levels (Ho & Dimitroupulos, 2010). As a result, management of this syndrome requires a comprehensive approach including different therapies and treatments (Eiholzer & Whitman, 2004).

In individuals of all ages, with and without PWS, physical activity (PA) is a key factor for

sustaining energy balance and weight maintenance (Eiholzer & Whitman, 2004; Fulton, Garg, Galuska, Rattay, & Caspersen, 2004; Haskell et al., 2007; Mullins & Vogl-Maier, 1987). Physical activity stimulates muscle growth, motor coordination and balance (Eiholzer et al., 2003; Haskell et al., 2007). Physical activity offers psychological benefits to individuals such as improved self-esteem (Altintas & Asci, 2008) and quality of life (Schwimmer, Burwinkle, & Varni, 2003). Previous reports show that school-day levels of PA in children with PWS are lower than in children without the syndrome; however these differences are less when overall daily PA is considered (van den Berg-Emons, Festen, Hokken-Koelega, Bussmann, & Starm, 2008). Similar to children with PWS, adults with PWS present less spontaneous PA even in comparison to obese adults without the syndrome (Butler, Theodoro, Bittel & Donelly, 2007).

Parents and caregivers play a pivotal role in children's PA, through example (e.g., the parent engages in regular PA) and through the provision of opportunities (e.g., enrolling their children in organized sports activities, walking with their child to school) (Bauer, Nelson, Boutelle, & Neumark-Sztainer, 2008). Challenges and barriers to participation in PA in children and adults are varied (Sherwood & Jeffery, 2000). Because of the wide spectrum in cognitive profile and behavior in PWS (Ho & Dimitropoulos, 2010), parents of these individuals with PWS may face other facilitators and challenges to PA participation.

The purpose of this study is to present, from the parents'/caregivers' perspective, descriptive PA data on individuals with PWS, as well as the perceived barriers and facilitators for PA in this population. We believe that identification of barriers and facilitators, such as parents' perceived benefits of their child's engagement in PA, can help define future intervention strategies for children with PWS.

Methods
Participants
Participants were recruited mostly from the Prader-Willi California Foundation registry.

Four hundred surveys were mailed out to parents in the registry or distributed at PWS related functions. The response rate was approximately 25 % which provided an initial n=104. As the results from surveys containing children younger than five years old (n=14) were excluded, the analytic sample consisted of 90 participants (n=90). All participants had one child who had been medically diagnosed with PWS (diagnosis letter from physician not provided). Only one parent/caregiver from each household was considered for the survey.

Procedures
The survey was mailed to all 375 members of the Prader-Willi California Foundation (PWCF) registry with an information sheet about the purpose of the project. An advertisement about the study was also placed in the Prader-Willi Syndrome USA Association website. PWCF members were invited to complete the self-report survey and mail it back to the researchers using a pre-addressed stamped envelope. Parents not belonging to PWCF but were interested in participating contacted the principal investigators via email and requested survey materials. Participants who returned the completed survey received a $10 gift card by mail. Survey responses were entered into a password-protected computer, with identifying information removed. Once the data was entered, it was compared to the original surveys to ensure accuracy of data entry by a different researcher. The study protocol was approved by the Institutional Review Board of the investigators' affiliation as well as sponsoring agency.

Measures

Demographic Characteristics.
Parent participants reported their age, sex, ethnicity, language spoken at home, employment status, and educational level.

Levels of PA among Parents.
By answering two separate multiple choice questions, parents reported the number of days in the past week that they engaged in PA, as well as the usual duration of each PA session (in minutes).

Levels of PA among individuals with PWS and other activities.

First, parents reported the number of times per week and the number of minutes per day that each of their children (including their child with PWS) engaged in structured and recreational PA. Then, parents were asked to report how many minutes and times per week their child with PWS engaged in a list of activities. The list of activities was developed from activities previously used on other children PA questionnaires (Gilmer, Speck, Bradley, Harrell, & Belyea, 1996) as well as activities reported in four group interviews with parents of youth with PWS from Southern California. Activities were then classified using metabolic equivalents (MET) into moderate intensity (≥3.8-5.9 METS), vigorous intensity (≥6 METS), and bone-strengthening based on the compendium of PA (Aimsworth et al., 2000; Harrell et al., 2005). METs were used to categorize activities, but not to estimate energy expenditure. Because individuals with PWS have less lean mass, it is likely their energy expenditure for physical activities is lower than normal. Therefore, actual MET expenditure may not be reliably estimated based upon the survey measures we utilized (M. G. Butler et al., 2007). However, although the absolute MET value for activities in individuals with PWS may be different, the relative difference in MET values among the activities may remain relatively constant, allowing for their use to classify activities into moderate or vigorous intensity. Parents also reported whether or not their child with PWS has ever engaged in the following therapies: physical, aquatic, horse, speech, and occupational. In addition, parents reported if their child attended learning disability or psychological services, or was under growth hormone replacement therapy, dietary management, and structured PA.

Perceived benefits and barriers. Participants were asked the extent to which the following reasons would *encourage* them to enroll their child in a PA program: [their child] benefitting from the program, improving motor skills and balance, improving stamina/strength, and socially interacting with other children, [the parent participant] talking to doctors and other experts, receiving increased opportunities for participation in other programs, and meeting other parents of children with PWS. Participants were also asked the extent to which the following reasons would discourage them to enroll their child in a PA program: the time commitment, traveling to the site, and not getting along/relating to the program staff. Responses to all items ranged from 1 (Strongly Disagree) to 5 (Strongly Agree). The reasons were derived from feedback obtained in four group interviews with 20 parents of youth with PWS.

Data analysis

Participants were stratified into the following age groups: children 5 to 10 years (n=28), adolescents 11 to 17 years old (n=27) and adults 18 years old and over (n=35). These age – groups were chosen because they represent physical and developmental milestones. Descriptive statistics (frequencies and percentages, means and standard deviations where appropriate) were calculated for parent and sibling characteristics (parent's age, gender, employment, education level, marital status, PA frequency and duration, and siblings' participation in structured and recreational PA in minutes/week), and characteristics of the child with PWS (gender, age group, and lifetime participation in treatments and therapies).

For the child with PWS, frequencies and percentages were calculated for weekly participation (yes or no) for the various PAs that were categorized as moderate, vigorous, or bone-strengthening. Chi-square tests were conducted to determine whether percentages for these PA variables differed according to the age of the child with PWS. Means and standard deviations were calculated for the average number of minutes/week that the child with PWS engaged in each of the moderate, vigorous and bone strengthening PAs, as well as the total number of minutes for each of these three PA categories. ANOVAs were performed to determine age group differences on these PA variables. Last, ANOVA tests were performed to determine whether responses for each of the perceived benefit, perceived barrier, and perceived need variables differed among the three age groups.

Results

Demographic Characteristics

Parent and sibling characteristics. Mean age of parent participants was 49.2 ± 10.5 years. The majority of the parent participants was female (86.7%), Caucasian (70.8%), and spoke English at home (92.2%). Sixty-three percent of the sample was employed; of them 26.8% worked over 40 hours per week. The education level of participants was high, as 26.3% had a college degree, and 35.1% had at least some graduate school or a graduate/professional degree. Most participants (82.2%) were married. Only six parents (6.7%) reported that they did not engage in any past week PA; 31.4% engaged in PA 1 to 2 times in the past week, 35.6% 3 to 4 times in the past week, 17.8% 5 to 6 times in the past week, and five of them (5.6%) every day. Among those who did engage in PA (n=84), 60.7% reported an average duration of at least 30 minutes per PA session. Only 18 parents (20%) of the entire sample engaged in PA at least 5 times per week for 30 minutes or more each time. Parents' PA did not vary by age of their child with PWS (5 to 10 years, 11 to 17 years, and 18+ years).

Table 1

Lifetime participation in treatments
and therapies to manage PWS Symptoms
(n = 90)

Treatment	N	%
Learning disability services	46	51.7
Psychologist	35	39.3
Growth hormone replacement	51	57.3
Dietary management	70	79.5
Structured PA	54	61.4
Therapy	N	%
Physical	55	61.1
Aquatic	27	30.3
Horse	27	30.3
Speech	76	85.4
Occupational	56	62.9

The children with PWS had an average of 1 ± 1 sibling. Siblings participated in 379 ± 614 minutes per week of recreational PA and 255 ± 370 minutes per week of structured PA. Siblings' PA did not vary by age group that the child with PWS belonged to.

Characteristics of the individuals with PWS. Forty-eight of the individuals with PWS (53.3%) were male. There were twenty-eight children (31.1%) ages 5 to 10 years old, 27 adolescents (30.0%) ages 11 to 17 years old, and 35 adults (38.9%) ages 18 to 49 years old (mean 28 ± 9 years). Frequencies and percentages for the types of treatments and therapies to help manage PWS symptoms that the child had participated in are presented in Table 1. The majority (60%) of individuals with PWS engaged in both PA and physical therapy.

Moderate, Vigorous, and Bone Strengthening PA among individuals with PWS

Moderate PA (MPA).
There were no age group differences in past week participation (yes or no) in any of the MPAs, all of which are listed in Table 2. However, there was an age group difference in the total number of minutes per week spent in MPA: adolescents engaged in more MPA than children (262 ± 222 vs. 100 ± 76 minutes per week; $p<0.05$).

Vigorous PA (VPA).
There were age group differences for past week participation (yes or no) for some activities. Running was more common in adolescents than in children or adults (37.0% vs. 17.9% and 8.6%, respectively; $p<0.05$). Swimming ($p<0.05$) and jumping on a trampoline ($p<0.01$) were significantly more common in children (64.3% and 25.0%, respectively) compared to the other groups. In addition, children engaged in more VPA than adults 18 years and above (239 ± 361 vs. 70 ± 161 minutes per week, $p<0.05$). See Table 3. Relatively few individuals with PWS engaged in competitive sports (8 out of 90 children, 8.9%) and martial arts (3 out of 90 children, 3.3%).

Muscle/bone strengthening PA.
With the exception of playing games (e.g., tug of war), there were no age group differences for past week engagement (yes or no) in

Table 2

Frequency and average number of minutes per week of moderate physical activity (MPA) in individuals with PWS by age group (n= 90)

	5-10 years (n = 28)	11-17 years (n = 27)	18+ years (n = 35)	
Moderate PA	f (% yes)	f (% yes)	f (% yes)	p
Hiking	2 (7.1)	3 (11.1)	1 (3.3)	0.52
Walking	21 (75.0)	22 (81.5)	22 (62.9)	0.25
Bicycle/Tricycle riding	8 (28.6)	7 (25.9)	4 (11.4)	0.19
Exercise video games	3 (10.7)	6 (22.2)	5 (14.3)	0.48
Housework/Yardwork	9 (32.1)	14 (51.9)	16 (45.7)	0.32
Other Moderate PA[1]	4 (14.8)	4 (15.4)	5 (19.2)	0.90
	Mean (SD)	Mean (SD)	Mean (SD)	p
Total Moderate PA (min).	100.0 (76) [a]	262 (222) [b]	192 (225) [ab]	0.01**

**p < .01.

Note. Groups containing the same superscript letters have homogenous values for the given variable.
[1] Responses from individual participants included climbing stairs (n = 3), using cardio equipment at a moderate pace (n = 3), bowling (n = 1), horseback riding (n = 2), *Wii Sports* (n = 1), slow walking (n = 1), hula-hoop (n = 1), and moderate-level outdoor play (n = 1).

Table 3

Frequency and average number of minutes per week of vigorous physical activity (VPA) in individuals with PWS by age-group (n= 90)

	5-10 years (n = 28)	11-17 years (n = 27)	18+ years (n = 35)	
Vigorous PA	f (% yes)	f (% yes)	f (% yes)	p
Jump rope	2 (7.1)	1 (3.7)	0 (0.0)	0.29
Running	5 (17.9)	10 (37.0)	3 (8.6)	0.02*
Swimming	18 (64.3)	12 (44.4)	11 (31.4)	0.03*
Martial arts	1 (3.6)	2 (7.4)	0 (0.0)	0.27
Competitive sports	2 (7.1)	3 (11.1)	3 (8.6)	0.87
Trampoline	7 (25.0)	3 (11.1)	0 (0.0)	0.01**
Dancing	5 (17.9)	6 (22.2)	2 (5.7)	0.15
Other Vigorous PA[1]	5 (17.9)	2 (7.7)	2 (6.9)	0.34
	Mean (SD)	Mean (SD)	Mean (SD)	p
Total Vigorous PA	239 (361) [a]	146 (130) [ab]	70 (161) [b]	0.02*

*p < .05; **p < .01.

Note. Groups containing the same superscript letters have homogenous values for the given variable.
[1] Responses from individual participants included vigorous outdoor play (n = 3), using cardio equipment vigorously (n = 2), gymnastics (n = 2), playing basketball (n = 1), and playing tennis (n = 10).

muscle/bone strengthening PAs. Playing games was significantly more common in children than in adolescents or adults (25.0% vs. 14.8% and 2.9% respectively, p<0.05). There was no age

group difference in the total number of minutes per week individuals engaged in muscle/bone strengthening PA. See Table 4.

Parental Perceptions of Enrolling their Child with PWS in a PA Program
Perceived benefits. The majority of parent participants "agreed" to all of the perceived benefits listed. The percentage of "strongly agree" responses were higher for their child: (1) benefitting from the program (77.0%), (2) improving motor skills and balance (78.2%), and

(3) improving stamina and strength (74.4%). The percentage of "strongly agree" responses were lower for meeting other parents of children with PWS (37.9%). ANOVA tests showed that improving motor skills and balance as a benefit from PA varied by age group ($F_{(2, 84)}$= 3.91, p=0.02). On a scale of 1 to 5, mean scores were the following: 4.9 ± 0.3 for children, 4.8 ± 0.5 for adolescents, and 4.5 ± 0.8 for adults. Parents of adults had significantly lower scores than parents with children and adolescents.

Table 4

Frequency and average number of minutes per week of muscle/bone strengthening physical activity in individuals with PWS by age group (n=90)

	5-10 years (n = 28)	11-17 years (n = 27)	18+ years (n = 35)	p
Muscle/Bone Strengthening PA				
Games (e.g., tug-of-war)	7 (25.0)	4 (14.8)	1 (2.9)	0.04*
Push-ups/Pull-ups	3 (10.7)	2 (7.4)	0 (0.0)	0.16
Rope climbing	1 (3.6)	0 (0.0)	0 (0.0)	0.33
Sit-ups	2 (7.1)	2 (7.4)	2 (5.7)	0.96
Resistance exercises	2 (7.1)	6 (22.2)	5 (14.3)	0.28
Other	7 (25.0)	3 (11.1)	2 (6.9)	0.13
	Mean (SD)	Mean (SD)	Mean (SD)	p
Total Muscle/Bone Strengthening	41 (65)	59 (129)	30 (94)	0.52

*p < .05; **p < .01.
Note. Groups containing the same superscript letters have homogenous values for the given variable.
[1] Responses from individual participants included physical therapy (n = 2), climbing (e.g., indoor rock climbing, n = 2), yoga (n = 1), circuit training (n = 1), core strengthening (n = 1), stretching (n = 1), and crawling (n = 1).

Perceived barriers.
Less than 40% of parents either "agreed" or "strongly agreed" that time commitment and travel to the program site would be a barrier to participating in a program. Only 15.1% of respondents "agreed" or "strongly agreed" that not getting along with program facilitators would be a barrier. ANOVA tests indicated that parents' perceived barriers did not vary by the age of their child (p> .05).

Perceived needs.
Over 70.1% of parents either "agreed" or "strongly agreed" that they needed more

financial support, and over one half answered similarly for needing more time out of their busy schedule (54.7%) and more equipment (51.2%). ANOVA tests indicated that parents' perceived need for encouragement (1 to 5 scale) varied significantly by age group. Parents of 5-10 year old children had a higher need for encouragement compared to those of adolescents or adults (3.7 ± 1.3 vs. 2.9± 1.3 and 2.8 ± 1.5, respectively; ($F_{(2, 84)}$= 3.47, p=0.04). Non-significant trends were found for parents' perceived need of financial support (p=0.07) and time out of their busy schedule (p=0.06). Specifically, parents of children who were 18

years and older reported a higher need of financial support compared to parents of children or adolescents (2.6 ± 1.3 vs. 2.0 ±1.2 and 2.0 ± 1.1, respectively; ($F_{(2, 84)}$= 2.81, p=0.10). Furthermore, to a near significant level, parents of children reported less need for time out of their busy schedule compared to parents of adolescents or adults (2.2 ± 1.2 vs. 3.0 ±1.3 and 2.9 ± 1.4, respectively; ($F_{(2, 81)}$= 2.87, p=0.07).

Discussion

This study is unique because (1) we obtained a relatively large sample of parents with children who have PWS, (2) the individuals of PWS being studied included young children, adolescents, as well as adults, and (3) we gathered data, from the parents' perspective, on the PA patterns of their child with PWS, as well as the parents' perceived benefits, barriers, and facilitators for engaging their child with PWS in PA. The findings of this study suggest that adolescents with PWS engage in more moderate intensity PA than younger children. Conversely, children ages 5-10 years appear to be engaged in more vigorous PA during the week compared to adults. The trend we observed is similar to PA patterns in children and adolescents who do not have PWS (Bradley, McMurray, Harrell, & Deng, 2000; Trost et al., 2002). Trost and colleagues found that as children progressed from elementary to high school there was a consistent decrease in time spent in vigorous PA during the week (Trost et al., 2002). Similarly, Bradley et al. (Bradley et al., 2000) showed that as children progressed from elementary to middle school their choices for sedentary and more moderate intensity activities increased.

We found that common activities among individuals with PWS were walking and yard work. Walking is also the most common activity in adults in the United States (Centers for Disease Control and Prevention, 2012), as well as with youth with PWS (van den Berg-Emons et al., 2008). European children ages 6-12 with PWS have been reported to cover about 12.6 km in three days (Schlumpf et al., 2006). We also found that very few individuals with PWS participated in competitive sports as

indicated in Table 3 (11.1% at most), which is in sharp contrast to the general population of children in California, where approximately 55.5% of children ages 10-11, 59.2% of children ages 12-14, and 47.4% of children ages 15-17 years old participate in sports team or taken sport lessons. It is possible that this lack of participation in organized sports relates to physical, developmental and cognitive characteristics of individuals with PWS. However, participation in organized sports should not be discouraged, as sports provide a social atmosphere which may be good for development of relationships with peers, contribute to character building, and improved self-esteem (Patel & Greydanus, 2010). Sports participation is highly encouraged in other people with intellectual or physical disabilities; it is a matter of finding the right activity and setting for a person with PWS (Patel & Greydanus, 2010).

A noteworthy finding of this study is that adults with PWS spent half of the time on bone or muscle strengthening activities (about 30 minutes per week) compared to adolescents or children. Strengthening activities are recommended for the general population at least twice a week and for youth 2-3 times per week (Faigenbaum et al., 2009). In people with PWS strengthening activities are particularly important for increasing and sustaining muscle mass. Increased muscle mass allows for more stamina to carry out everyday activities and improving overall quality of life (Eiholzer et al., 2003; Whitman, Myers, Carrel, & Allen, 2002). In addition, lean mass is more metabolically active than adipose tissue, thus a larger muscle mass contributes to sustaining energy balance (McArdle, Katch, & Katch, 2007). It appears that PA interventions, particularly those that involve muscle and bone strengthening, are particularly important for individuals with PWS, because independent of their body size, they have less muscle mass compared to the general population (Brambilla et al., 1997; Rubin, Wright, Haqq, Castner, & Judelson, 2012).

A large proportion of responding parents indicated that their child benefitted from participating in a PA program and improving

motor skills and balance was important to them. These results support the fact that 61% of the sample had been enrolled in physical therapy sessions during some period of their lifetime. Furthermore, 74.4% of parents in this study believed that a major benefit of engaging their child with PWS in PA is to improve his/her stamina and strength. The parents' beliefs are consistent with the findings of three exercise interventions that demonstrated increased spontaneous PA, muscle endurance, and aerobic capacity in individuals with PWS (Eiholzer et al., 2003; Schlumpf et al., 2006; Silverthorn & Hornak, 1993). Parents also indicated that engaging in PA had a social component that was beneficial to their child. Similar to other populations (Sherwood & Jeffery, 2000), time and financial support appear to be main barriers to participating in PA in PWS.

Limitations

A limitation of the study is the survey method, in that PA data being self-reported by parents are less reliable and valid than observation or direct monitoring. As previously indicated, we used MET values from individuals without PWS and it is likely that MET values for different activities are lower in those with PWS because of their lower amount of lean mass. In addition, the sample may not necessarily represent the entire population of parents of individuals with PWS, as our sample of parents reported a relatively high level of formal education, and the majority of them lived in the state of California.

Implications of the Results
The results of this study show that most individuals with PWS are engaged in PA of moderate intensity. However, participation in vigorous PA decreases with age. Vigorous PA
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provides health-related (D. A. Rubin, McMurray, & Harrell, 2008) and fitness-related benefits above and beyond the ones of moderate intensity PA (Haskell et al., 2007). Similarly, with increased age there is less participation in bone and strengthening activities. It is recommended that caregivers and health care providers continue to emphasize the role of vigorous and strengthening PA as individual with PWS grows into adulthood.

Similar to parents in the general population, parents of individuals with PWS are encouraged to enroll their child in a PA program if they perceive that specific beneficial outcomes (e.g., improved motor skills and balance) may occur. However, parents of younger children may need additional encouragement to have their child participate in all types of PA, perhaps because they are still learning to cope with the challenges that a diagnosis of PWS involves. Parents' perceptions of their child with PWS engaging in PA are particularly important to consider, because parents are most often the lifetime caregivers and decision makers for the PWS child. Hence, parents' perceptions regarding the benefits and barrier of their child engaging in PA can significantly impact the PA levels of their children.

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APPENDIX D

Manuscript draft and acceptance e-mail: Rubin, D.A., Cano-Sokoloff, N., Castner, D.M., Judelson, D.A., Wright, P., Duran, A., Haqq, A.M. Update on body composition and bone density in children with Prader-Willi Syndrome. *Hormone Research in Pediatrics,* 2012.

"Update on body composition and bone density in children with Prader-Willi Syndrome"

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List of words: congenital obesity, bone mineral content, lean mass, adiposity, growth

Short title: Update on body composition in Prader-Willi Syndrome

1 **Abstract**

2 Aim: To compare body composition in children with Prader-Willi Syndrome (PWS) not naïve to

3 growth hormone (GH) with obese and lean controls.

4 Methods: Participants included 12 children with PWS, 12 children with obesity (body fat % >95th

5 percentile for age and sex), and 12 lean children (body fat % <85th percentile for age and sex)

6 matched by age and height. Fat mass, lean mass, bone mineral content (BMC), bone mineral density

7 (BMD), and BMD z-score for total body, hips, and lumbar spine obtained through dual x-ray

8 absorptiometry.

9 Results: PWS had higher fat percentage in the legs ($p=0.04$) but similar leg fat mass ($p=1.00$)

10 compared to obese. PWS exhibited lower lean mass in the body ($p=0.04$) and legs ($p=0.02$) than

11 obese, but similar to lean ($p=1.00$ and $p=0.89$, respectively). PWS had lower hip BMC ($p<0.01$),

12 BMD ($p<0.01$) and BMD z-score ($p<0.01$) compared to obese but similar to lean. No other

13 differences were found between PWS and obese ($p>0.05$ for all).

14 Conclusions: Children with PWS not naïve to GH present differences in fat and lean mass distribution

15 compared to obese controls. Bone mineral content and density appears unaffected by PWS, except at

16 the hips.

1 **Introduction**

2 Lean mass, fat mass, and bone density are regulated by intrinsic factors (e.g. growth hormone [GH],

3 thyroid hormone, and leptin) and extrinsic factors (e.g. physical activity and diet) [1]. During

4 childhood, mechanical loading provides a sustained skeletal benefit that endures into adulthood [2].

5 Children who are obese may experience extra mechanical stress because of the extra body mass they

6 carry. However, obesity in childhood may be detrimental long-term to bone growth and development,

7 specifically when fat is deposited in the abdominal area [3, 4].

8 Prader-Willi Syndrome (PWS) is the best-characterized genetic form of childhood obesity; PWS

9 results from an absence of expression of the paternal chromosome 15q11-q13 [5]. Overall, individuals

10 with PWS present a low lean mass and a relatively high body fat content [5]. Adults with PWS not on

11 GH (especially males) display high fat mass in the legs [6]. Adults with PWS also present low bone

12 mineral density (BMD) [7] due to a low bone mineral content (BMC) [8] and a high prevalence (60-

13 90%) of osteoporosis [9]. Factors such as lack of growth hormone, hypogonadism, and reduced

14 spontaneous activity have been associated with this unique body composition [6-8, 10]. A trend

15 towards low BMD has also been suggested in children with PWS [11].

16 Growth hormone replacement therapy (GHRT) is part of the complex management of PWS.

17 Treatment with GHRT improves body composition by increasing lean mass and decreasing fat mass

18 [12,13]. However, increases in BMD with GHRT have been shown in one study [12], while three

19 studies show no change in BMD standard deviation scores or z-scores for the spine and total body

20 [14-16]. There are no studies that evaluated bone density for the hips.

21 Although several studies have presented body composition characteristics in individuals with PWS,

22 no studies have focused on children with PWS at the earlier stages of development critical to peak

1   bone mass. Moreover, studies comparing those with PWS to lean or obese controls have included

2   cohorts of subjects with PWS of a wide range of age, and have lacked uniformity in their selection of

3   participants for use of GHRT. Therefore, this study compared body composition in children with

4   PWS who have been on GHRT for at least two years to obese and lean controls matched for age and

5   height.

6   **Methods**

7   *Participants*

8   Participants included 12 children with PWS, 12 children with non-syndromic obesity (body fat

9   percentage $> 95^{th}$ percentile for age and sex), and 12 lean children (body fat percentage $< 85^{th}$

10 percentile for age and sex) [17] matched for age and height to control for parameters influencing bone

11 development [18]. Participants' age ranged from 8 to 11 years old. PWS status was documented by

12 appropriate molecular and cytogenetic testing (i.e., chromosomes, FISH 15, DNA methylation, and/or

13 DNA polymorphism studies). Eight participants with PWS had uniparental deletion, while four had an

14 undetermined cause of the syndrome. In the group with PWS, one participant had diabetes, one had

15 asthma, two had sleep apnea, four had seizures, two had hip dysplasia, and two had scoliosis. All the

16 children with PWS had received GHRT at least for two years at the time of the study. Two

17 participants had also received a testosterone injection in the past, two currently used Albuterol, and

18 one used inhaled glucocorticoids. Eleven of the 12 subjects with PWS were or had been receiving

19 physical therapy. There were no controls with insulin resistance, type 2 diabetes mellitus or other

20 metabolic disease.

21 *Procedures*

1   The data used for the present study was collected as part of ongoing studies approved by the

2   California State University Fullerton Institutional Review Board, the Children's Hospital of Orange

3   County and the U.S. Army Material and Research Command. Parents signed the approved informed

4   consent and youth signed the informed assent. Measurements included body mass, stature, body mass

5   index (BMI), and body composition. Body mass was obtained using a digital scale (ES200L, Ohaus,

6   Pinewood, NJ) while wearing no shoes and the subject wearing a t-shirt and shorts. Stature was

7   measured at the end of inhalation using a wall-mounted stadiometer (Seca, Ontario, CA). Body mass

8   index (BMI) was computed by dividing body mass in kg by the stature in meters, squared. Tanner

9   stage was either determined by a physician (children with PWS) or by a previously validated

10 questionnaire [19] filled out by children and their parents (obese and lean controls).

11 *Body composition measurements*

12 All measurements were assessed by dual x-ray absorptiometry (Lunar Prodigy Advance, GE,

13 Healthcare, Madison, WI). For those participating girls who had their first menses, a urine pregnancy

14 test was completed prior to the body composition scan. No pregnant subjects participated in the study.

15 Quality assurance (each day scans were performed) and spine phantom scans (once per week) were

16 completed to ensure quality control of the instrument. Participants were positioned following

17 manufacturer's indications. Body fat and lean tissue were expressed both as mass in kg and as

18 percentage of body mass. The distribution of fat and lean mass was presented either as total, trunk,

19 arms and legs. Body fat distribution was also assessed as android, which refers to the localization of

20 excess fat mainly in the upper body, or gynoid, which refers to the localization of excess fat mainly in

21 the lower body. Bone measurements were determined for the total body without head (total)[20],

22 lumbar spine (L1-L4), and hip (mean as well as femoral neck). Measurements were presented as

1 BMC in grams (g), BMD in g/cm^2, and BMD z-scores. For participants in whom there were no

2 available reference data because of ethnicity, the default ethnic denomination of Caucasian was used.

3 *Data analysis*

4 Subject descriptive characteristics were compared among groups using one-way analysis of variance.

5 Multivariate analysis of variance (MANOVA) was used to compare 1) lean and fat mass, and 2) bone

6 density among groups. Sex, pubertal stage and ethnicity were included as covariates, when

7 appropriate. If group differences were obtained, pairwise comparisons were conducted using the

8 Bonferroni adjustment. The significance level was chosen at 5%. Data were expressed as number (N),

9 percentage (%), mean (M), and standard deviation (SD).

10 **Results**

11 The characteristics of the participating children are shown in Table 1. Children with PWS and obese

12 controls had significantly higher body mass and BMI than lean controls. No other significant group

13 differences were observed.

14 *Body fat and lean mass parameters*

15 Table 2 presents fat and lean mass characteristics content. Children with PWS and obese controls had

16 higher total body, trunk, arms, and legs fat mass, fat percentages compared to lean controls. In terms

17 of body fat distribution, children with PWS also had higher percentage of fat in the legs than obese or

18 lean controls. Children with PWS had lower lean mass (both total and specifically in the legs)

19 compared to obese controls. There were no other statistically different measurements between those

20 with PWS and obese controls.

21 *Bone density*

1   Two participants with PWS showed co-morbidities that might independently influence BMD. Data

2   analyzed with and without these two subjects reached identical conclusions; thus, the following data

3   include all participants. Bone density values of total body, lumbar spine and hip are displayed in

4   Table 3. There were no significant differences in total body BMC or BMD among groups. The total

5   body BMD z-score was lower in children with PWS and lean controls compared to obese controls.

6   There were no significant differences in lumbar spine BMC, BMD and BMD z-scores among the

7   groups. In contrast, children with PWS had lower values in hip BMC, BMD and BMD z-score than

8   obese controls; however, no significant differences were seen between PWS and lean controls.

9   Similarly, obese controls exhibited a higher femoral neck BMC, BMD and z-score than either PWS or

10   lean controls.

11   **Discussion**

12   This study evaluated differences in fat and lean mass and their distribution in growing children with

13   PWS who had been on GHRT. No differences in fat mass or its distribution (except for the legs) were

14   demonstrated between these children with PWS and obese controls. Of interest, the lower lean mass

15   shown in adults with PWS [10] was not seen in children who were not GH naïve when compared to

16   lean controls. Osteopenia is a clinical concern in adults with PWS. This study shows that developing

17   children with PWS appear to have normal bone mineral density. However, the lower bone

18   measurement values at the hip and femoral neck in PWS compared to non-syndromic obese warrant

19   attention.

20   *Fat and lean mass distribution*

21   This study found no major site-specific differences in body fat (with the exception of the legs)

22   between those with PWS and obese controls. In contrast, a previous study in GH naïve children and

1   adults with PWS demonstrated a higher fat mass and percentage compared to obese controls [10]. In

2   addition, less lean mass in individuals with PWS has been demonstrated in early stages of

3   development with this characteristic persisting into adulthood [6,10,11]. The present study shows

4   lower total body and legs lean mass in PWS compared to obese children but similar to the lean

5   controls. Possibly differences in sex hormones contribute to body composition characteristics in PWS

6   [6,10]. However, the increased fat percentage in the legs in those with PWS shown by this study and

7   others in adults [6,10] could be explained by hypotonia-induced decreases in leg lean mass.

8   Individuals with PWS typically present with diminished spontaneous activity and ambulation [21],

9   providing a reduced stimulus for lower body muscle accretion [11, 22]. Our data suggest that the

10   higher leg fat percentages noted compared to obese controls resulted from the combination of this

11   lower lean mass and similar fat mass.

12   *Bone measurements*

13   Most previous studies in PWS have compared bone parameters for the whole body and the spine,

14   demonstrating lower BMD particularly in adults [7, 8], and some in children [11]. Our results show

15   that children with PWS who have been on GHRT present no differences either in BMC or BMD (total

16   body or at the lumbar spine) when compared to obese or lean controls. In contrast, the present study

17   found significantly lower BMC, BMD and corresponding standardized scores at both the femoral

18   neck and the hip in those with PWS compared to obese controls, but no differences between PWS and

19   lean controls.

20   Different authors speculated about possible factors being responsible for the lower BMD in adults

21   with PWS such as GH and sex hormones deficiency, as well as the hypotonia which can affect the

22   spontaneous activity [7, 8]. We think that the lesser amount of weight bearing physical activity may

23   best explain the reduced bone parameters in the hips of PWS when compared to obese controls [23].

1   Ambulatory children with muscular dystrophy presented a lower volume of weight bearing activity

2   and lower BMD in the proximal femur region but not in the spine compared to healthy controls [24].

3   Thus, the typically reduced volume of physical activity in PWS possibly contributes to a lower hip

4   BMD than obese controls. As lean controls and PWS had comparable lean mass, and lean mass is

5   closely associated with BMD in children [25], it makes sense that those with PWS had comparable

6   hip BMD to lean controls.

7   Although our study had age-and height-matched groups, it would have been ideal to include a

8   comparison group of children with PWS who were GH naïve. The inclusion of this 4th group would

9   have clearly distinguished if differences we see in these youngsters with PWS might be related to

10   GHRT or perhaps to other management factors such as diet or physical activity [5,12]. Nonetheless,

11   this study shows novel data in children with PWS having lower BMC and BMD in the hips compared

12   to obese counterparts but similar to lean. This finding is of clinical relevance since optimization of

13   bone mass at entry into adulthood may reduce the proportion of fractures experienced later in life

14   [26]. Given that these children are going through the period of growth and development, it seems vital

15   that strategies are set forth to maximize the chances of attaining the highest possible peak BMD (e.g.

16   through nutrition and increased weight bearing physical activity).

17   **Conclusion**

18   Our study results showed that some of the unique body composition characteristics of adults with

19   PWS such as higher fat mass and percentage compared to obese controls, or lower lean mass

20   compared to lean controls, were not present in children with PWS not GH naïve. We demonstrated

21   that children with PWS who were not GH naïve had similar fat deposition compared to non-

22   syndromic obese controls, except in their lower limbs. Additionally, our results showed that children

23   with PWS exhibited no differences in whole body or lumbar spine bone measurements compared to

1   other children. However, because of the lower bone parameters at the hip in PWS compared to obese

2   controls, strategies to maximize bone mineralization in PWS should be considered.

3   **Acknowledgements**

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Table 1: Demographics and anthropometrics of the children with PWS, children with non-syndromic obesity and lean controls presented as mean and standard deviation (M ± SD) or frequency

	PWS (N=12) M ± SD	Obese (N=12) M ± SD	Lean (N=12) M ± SD	*p*
Age (y)	10.2 ± 1.0	10.2 ± 1.0	9.9 ± 1.0	0.79
Sex (Males:Females)	7:5	7:5	4:8	
Pubertal Stage (N)				
I	2	4	5	
II	3	1	3	
III	6	7	4	
IV	1	0	0	
Ethnicity (N)				
Asian	2	2	0	
Hispanic	4	8	7	
Non Hispanic White	4	1	5	
Other	0	1	0	
Body mass (kg)	52.3 ± 14.6*	56.4 ± 16.0*	34.5 ± 7.1	<0.01
Stature (cm)	143.0 ± 12.5	145.9 ± 8.1	143.9 ± 8.5	0.77
Height for age percentile	53.1 ± 34.6	68.0 ± 22.4	58.4 ± 22.6	0.40
BMI (kg/m^2)	25.4 ± 5.4*	26.0 ± 5.4*	16.4 ± 1.8	<0.01

* = different from lean; *p*<0.050

Table 2: Total and regional fat and lean mass distribution in children with PWS, children with non-syndromic obesity and lean controls presented as mean and standard deviation (M ± SD)

	PWS M ± SD	Obese M ± SD	Lean M ± SD	*p*
Total and Regional measures				
Body fat mass (kg)	24.4 ± 9.7*	24.4 ± 8.7*	6.7 ± 2.7	<0.01
Body fat (%)	47.6 ± 6.6*	42.8 ± 6.7*	20.1 ± 4.4	<0.01
Body lean mass (kg)	25.5 ± 5.3†	31.23 ± 6.6	26.4 ± 4.3	0.03
Android fat (%)	53.9 ± 6.8*	51.2 ± 7.6*	20.0 ± 7.3	<0.01
Gynoid fat (%)	54.6 ± 4.0*	50.1 ± 5.0*	32.3 ± 4.9	<0.01
Android-to-gynoid fat ratio	0.99 ± 0.07*	1.02 ± 0.11*	0.61 ± 0.14	<0.01
Trunk measures				
Fat mass (kg)	12.0 ± 5.3*	11.8 ± 4.5*	2.8 ± 1.6	<0.01
Fat (%)	47.2 ± 8.2*	43.6 ± 7.5*	18.9 ± 5.1	<0.01
Lean mass (kg)	12.2 ± 2.6	14.6 ± 3.3*	11.7 ± 2.1	0.03
Trunk-to-limbs fat mass ratio	1.0 ± 0.2*	1.0 ± 0.2*	0.8 ± 0.1	<0.01
Arm measures				
Fat mass (kg)	2.2 ± 1.0*	2.2 ± 0.9*	0.5 ± 0.3	<0.01
Fat (%)	45.7 ± 7.3*	40.8 ± 6.4*	16.3 ± 5.9	<0.01
Lean mass (kg)	2.4 ± 0.7	3.1 ± 0.9	2.9 ± 1.7	0.36
Lean-to-total body lean mass ratio	1.0 ± 0.1	1.0 ± 0.0	1.3 ± 0.8	0.34
Leg measures				
Fat mass (kg)	9.3 ± 3.4*	9.5 ± 3.4*	3.0 ± 1.0	<0.01

Fat (%)	$51.9 \pm 5.5^{*,\dagger}$	$46.1 \pm 6.4^{*,\ddagger}$	$24.7 \pm 4.2^{\dagger,\ddagger}$	<0.01
Lean mass (kg)	$8.3 \pm 1.9^{\dagger}$	10.7 ± 2.3	9.2 ± 1.9	0.02
Lean-to-total body lean mass ratio	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.1	0.13

* = different from lean, † = different from obese, ‡ = different from PWS; $p<0.050$

Table 3: Bone mineral content, bone mineral density, and bone mineral density z-scores of children with PWS, children with non-syndromic obesity and lean controls presented as mean and standard deviation (M ± SD)

	PWS M ± SD	Obese M ± SD	Lean M ± SD	*p*
Total (Head)				
BMC (g)	1206.775 ±352.215	1347.442 ± 354.466	1022.592 ± 297.403	0.17
BMD (g/cm^2)	0.825 ± 0.076	0.829 ± 0.207	0.807 ± 0.080	0.90
BMD z-score	0.6 ± 1.0[†]	1.4 ± 0.8	0.3 ± 0.9[†]	0.03
Lumbar spine				
BMC (g)	32.310 ± 8.220	32.966 ± 8.313	27.179 ± 7.146	0.49
BMD (g/cm^2)	0.876 ± 0.065	0.875 ± 0.128	0.795 ± 0.089	0.17
BMD z-score	0.8 ± 0.7	0.9 ± 0.8	0.1 ± 0.9	0.07
Hip				
Mean BMC (g)	17.853 ± 5.122[†]	23.211 ± 6.078	18.289 ± 5.281[†]	<0.01
Mean BMD (g/cm^2)	0.781 ± 0.126[†]	0.931 ± 0.117	0.808 ± 0.103[†]	<0.01
Mean BMD z-score	-0.2 ± 1.2[†]	1.3 ± 0.9	0.2 ± 1.0	<0.01
Femoral neck BMC (g)	2.933 ± 0.738[†]	3.749 ± 0.887	2.966 ± 0.637[†]	<0.01

Femoral neck BMD (g/cm^2)	$0.753 \pm 0.120^{\dagger}$	0.918 ± 0.124	$0.778 \pm 0.095^{\dagger}$	<0.01
Femoral neck BMD z-score	$-0.6 \pm 1.2^{\dagger}$	1.0 ± 1.0	$-0.4 \pm 0.8^{\dagger}$	<0.01

$\dagger$ = different from obese; *p*<0.050

Excellent news...will follow up shortly.
Dani

On 1/20/13 9:35 AM, "hrp@karger.com" <hrp@karger.com> wrote:

>
>Ms No.: 201212022
>Title: Update on body composition and bone density in children with
>Prader-Willi Syndrome
>
>Dear Dr. Rubin,
>
>Thank you for submitting your above mentioned manuscript to Hormone
>Research in Pediatrics.
>It has now been evaluated by our Editors and reviewers.
>
>We are pleased to inform you that your paper has been found suitable for
>publication, provided you can make the changes and amendments suggested
>by the referees.
>
>Below are remarks the reviewers have asked us to share with you:
>
>
>Reviewer 1
>This manuscript presents a comparison on body composition, fat and lean
>distribution and bone mineral density for Prader Willi Syndrome (PWS),
>Obese and Control children. Interesting comparisons have been shown in
>terms of lean and fat distribution in these 3 groups of children. It's
>especially interesting on terms of distribution in PWS children with
>Growth Hormone Replacement Therapy. That even with GHRT PWS children have
>different distribution of lean and fat compared to equally fat Obese
>children.
>
>Please check in Table 2 the % android and gynoid fat for the PWS
>children. The total adds up to more than 100%. If the values are correct
>then the results suggest something with the DXA tissue analysis that
>occurs with PWS patients, but not equally as large Obese patients. This
>would be worthy of comment in the discussion as to what might be the
>cause of the larger than 100% values, e.g. perhaps fat infiltration into
>lean tissue in PWS patients and DXA can't clearly separate lean and fat
>pixels
>
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>We look forward to hearing from you soon.
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APPENDIX E

Abstract: Castner, D.M., Rubin, D.A., Judelson, D.A., Haqq, A.M. Effects of adiposity and Prader-Willi Syndrome on post-exercise heart rate recovery, *Journal of Obesity, Special Issue: Interdisciplinary Aspects of Childhood Obesity and Physical Fitness*, In Review, 2012.

Title: Effects of adiposity and Prader-Willi Syndrome on post-exercise heart rate recovery

Authors: Diobel M. Castner[1], Daniela A. Rubin[1], Daniel J. Judelson[1], and Andrea M. Haqq[2]
[1]Department of Kinesiology, California State University, Fullerton, Fullerton, CA, USA
[2]Department of Pediatrics, University of Alberta, Edmonton, AB, Canada

Heart rate recovery (HRR) is an indicator of all-cause mortality in children and adults. We aimed to determine the effect of adiposity and Prader-Willi Syndrome (PWS), a congenital form of obesity, on HRR. Sixteen children of normal weight (NW=body fat % ≤85th percentile, 9.4±1.1y), 18 children with obesity (OB=body fat % >95th percentile, 9.3±1.1y), and 11 PWS youth (regardless of body fat %; 11.4±2.5y) completed peak and submaximal bike tests on separate visits. HRR was recorded one minute following peak and submaximal exercise. All groups displayed similar HRR from peak exercise, while NW (54 ± 16 beats) and OB(50 ± 12 beats) exhibited a significantly faster HRR from submaximal exercise than PWS (37 ± 14 beats).These data suggest excess adiposity does not influence HRR in children, but other factors such as low cardiovascular fitness and/or autonomic dysfunction might be more influential.

APPENDIX F

Invited Lecture: Diabetes and Endocrinology Research Center, Columbia University School of Medicine, New York, NY, April 2012.

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Thursday, April 26, 2012
Ruth Loos, Ph.D., Mount Sinai School of Medicine
New Insights in the Genetics of Obesity through Integrating 'OLD' Data
9am in Russ Berrie Pavilion, 1st Floor Auditorium

Thursday, April 19, 2012
Alexander Banks, Ph.D., Harvard Medical School
Modulation of PPAR-gamma and Insulin Sensitization: The Long March Toward Novel Therapeutics
9am in Russ Berrie Pavilion, 6th Floor

Link to Seminar

Thursday, April 12, 2012
Daniel Driscoll, MD, Ph.D.
Prader-Willi Syndrome: A Model System to Study Imprinting and Obesity
9am in Russ Berrie Pavilion, 6th Floor

Tuesday, April 10, 2012
Canceled: Dr. Alan Saltiel, University of Michigan
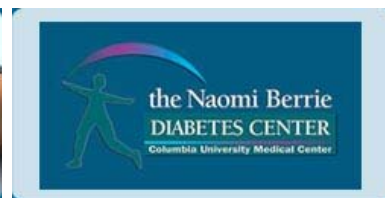Canceled: Inflammatory links between obesity and Type 2 diabetes
Canceled

Thursday, April 5, 2012
Lloyd E. Ratner, M.D., Columbia University
Pancreatic Transplantation
9am in Russ Berrie Pavilion, 1st Floor Auditorium

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APPENDIX G

Poster: American College of Sports Medicine Annual Meeting in San Francisco, CA, June 2012.

Evaluation of Body Fat Patterning in Children with Non-Syndromal and Syndromal Pediatric Obesity

Pamela Wright ◆ Daniela A. Rubin ◆ Diobel Mendoza-Castner ◆ Daniel A. Judelson

Fitness Assessment Laboratory ◆ Department of Kinesiology ◆ California State University, Fullerton

ABSTRACT

Prader-Willi Syndrome (PWS) is a genetic disorder resulting in excessive adiposity and reduced lean mass. Adults with PWS present differences in fat patterning (increased fat mass in the limbs) compared to non-syndromal obese adults who have increased fat mass in the trunk. In children, there is paucity of data. **Purpose:** To describe fat patterning in children with PWS as it compares to obese children without PWS. **Methods:** Eleven children with PWS and 42 obese (OB=body fat >95th percentile) children ages 8-11 y participated. Children underwent body mass, stature, waist circumference (WC) measurements and a total body dual x-ray absorptiometry scan. Body fat % was measured for total, trunk, gynoid, and android fat. Body mass index (BMI) was calculated. **Results:** Independent t-tests showed that PWS and OB had similar BMI (PWS: 24.7 ± 6.3 kg/m²; OB: 27.8 ± 9.4 kg/m²), and waist circumference (PWS: 80.4 ± 14.0 cm; OB: 88.6 ± 11.0 cm) (p>.05). Also, no significant differences were observed for total body fat % (PWS: 43.8 ± 8.0%; OB: 42.1 ± 8.0%), trunk fat (PWS: 43.9 ± 10.4%; OB: 44.1 ± 8.8%), gynoid fat (PWS: 53.2 ± 3.3%; OB: 49.8 ± 6.7%), and android fat (PWS: 53.6± 6.4%; OB: 51.0 ± 8.5%) (p >0.05 for all). **Discussion:** Previously, it has been shown that PWS adult males presented with higher gynoid body fat % than non-syndromal obese males with similar BMI. Our results suggest no differences in body fat patterning, particularly in the abdominal and limb regions, between PWS children and non-syndromal obese children with similar BMI and levels of body fat.

INTRODUCTION

Prader Willi Syndrome (PWS) is a genetic disorder characterized by hypotonia and excessive fat mass (1). Excessively high total adiposity, particularly in the abdominal region (android-type obesity) is a risk factor for heart disease, diabetes, and other chronic conditions (2). In non-syndromal obese children body fat appears to be more commonly distributed in the trunk region of the body (3). In PWS individuals however, adiposity is typically distributed in the hip region of the body (gynoid-type obesity) making them less susceptible to certain chronic conditions, such as insulin resistance (3,4). Studies have shown that when PWS individuals are treated with growth hormone replacement therapy (GHRT), they have increased lean mass and decreased fat mass (5,6). Previous reports have also found that adult males with PWS, who have not had GHRT, have differences in fat patterning compared to non-syndromal adults; namely, increased fat distribution in the legs (7). Because GHRT changes body composition in PWS, it is possible that the differences in body composition seen in adults are not present in children with PWS because currently, GHRT is part of the standard of care in children with PWS (1).

PURPOSE

To compare body fat patterning in children with PWS to non-syndromal obese children with similar BMI percentile.

METHODS

Children with PWS and non-syndromal obese children ages 8-11 years old participated in the study. Children without PWS were categorized as obese if their body fat was higher than the 95th percentile for age and sex (8). Children underwent anthropometric measurements following NHANES guidelines: body mass (kg), stature (cm), and waist circumference (WC) also in cm. Body mass index (BMI) was calculated from body weight and stature. Body composition was measured using dual x-ray absorptiometry scan (DXA) and total, trunk, gynoid, and android fat percentage (%) was derived from the DXA results.

RESULTS

There were 11 children with PWS and 42 non-syndromal obese children. The participant characteristics by group are presented in table 1. There were no significant differences ($p > 0.05$) in any of the anthropometrics measurements or body fat percentage measurements (Table 2) between the two groups. Particularly there were no differences between android and gynoid fat percentage (Figure 1) between obese children with and without PWS.

Table 1. Participant demographics, anthropometrics, and physiological characteristics (frequencies or mean ± SD))

	Obese	PWS
Frequency	42	11
Age (years)	10 ±1.0	10 ±1.2
Stature (cm)	143.0 ±13.0	142.0 ±15.0
Sex (F/M)	27/15	6/6
Body Mass (kg)	56.0 ± 13.3	50.5 ± 17.7
BMI (kg/m2)	27.8 ± 9.4	24.7 ± 6.3
WC (cm)	88.6 ± 11.0	80.4 ± 14.0
GHRT (yes/no)	Not applicable	10/1
Trunk Fat %	44.1 ± 8.8	43.9 ± 10.4

Figure 1. Android and gynoid body fat percentage in obese children with and without Prader-Willi Syndrome (PWS)
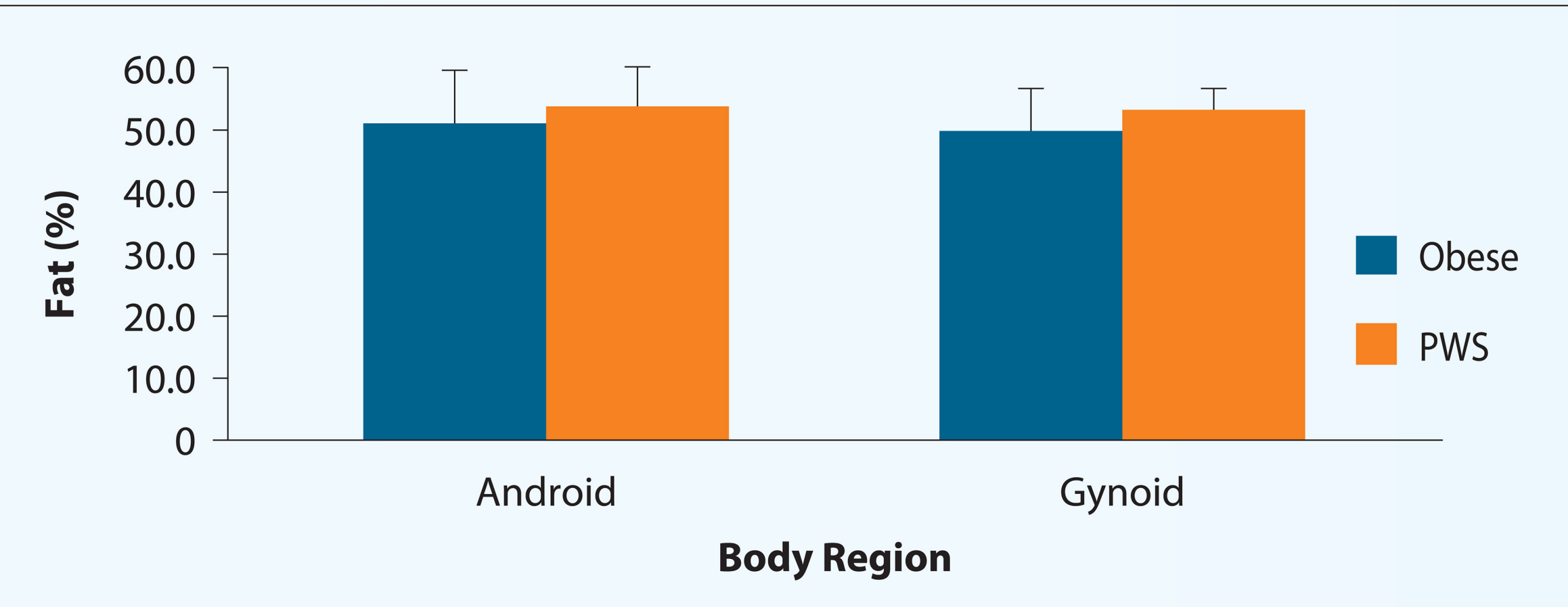
CONCLUSIONS

Previous studies suggest that adults with PWS present with increased fat mass in the hips compared to non-syndromal obese adults who store fat mass mostly in the trunk (7). Also, adults with PWS appear to present a higher body fat percentage compared to obese controls (3). In contrast, our results show similar fat percentage and distribution in children with PWS compared to those who have non-syndromal obesity. The differences between our findings and previous results in adults are most likely due to the majority of children in our study were on GHRT and the potential effect that GHRT may have on decreasing body fat mass (5,6). Another possible reason why fat patterning differences were not found between groups could be that all participants were 8-11 years old and many of them may not have gone through puberty. During puberty there is a marked increase in testosterone in boys, which plays a role in the android type shape compared to females who don't experience this hormonal change and tend to deposit fat in the hips (9). Our findings suggest that body fat percentage and distribution appears to be similar in children with PWS and those with non-syndromal obesity.

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ACKNOWLEDGEMENTS

APPENDIX H

Program: Rubin, D.A. (2012) Exercise in Children with Congenital Obesity (Prader-Willi Syndrome) and Non-Congenital Obesity. Special event presentation at American College of Sports Medicine Annual Meeting in San Francisco, CA.

478 **3:45 PM - 4:10 PM**
Exercise in Children with Congenital Obesity (Prader-Willis Syndrome) and Non-Congenital Obesity
Daniela Rubin. *California State University Fullerton, Fullerton, CA.*
(No relationships reported)

479 **4:10 PM - 4:35 PM**
Exercise in Children with Severe Burns
Elisabet Børsheim. *UTMB/Shriners Hospitals for Children, Galveston, TX.*
(No relationships reported)

480 **4:35 PM - 5:00 PM**
Exercise, Inflammation and Oxidative Stress in Children with Diabetes
Pietro Galassetti. *UC-Irvine, Irvine, CA.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-44 Symposium **- Behavioral Compensation to Exercise: Do We Eat More and Do Less?**
FRIDAY, JUNE 1, 2012 3:15 PM - 5:15 PM
ROOM: 3000

481 **3:15 PM - 3:20 PM**
Chair: Barry Braun, FACSM. *University of Massachusetts, Amherst, MA.*
(No relationships reported)

482 **3:20 PM - 3:45 PM**
Do Sedentary Behavior and Habitual Physical Activity Influence Responsiveness to Exercise Training
Sarah Kozey Keadle. *University of Massachusetts, Amherts, MA.*
(No relationships reported)

483 **3:45 PM - 4:10 PM**
The Effects of Exercise on Non-exercise Activity and Energy Expenditure
Edward Melanson, FACSM. *University of Colorado Denver Anschutz Med Campus, Aurora, CO.*
(No relationships reported)

484 **4:10 PM - 4:35 PM**
The Effects of Exercise on Ad Libitum Energy and Macronutrient Intake
Joseph E. Donnelly, FACSM. *University of Kansas Medical Center, Kansas City, KS.*
(No relationships reported)

485 **4:35 PM - 5:00 PM**
The Interaction Between Exercise and Appetite: Hedonic and Homeostatic Compensatory Responses
Neil King. *Queensland University of Technology, Brisbane, Australia.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-45 Symposium **- Exercise Induced Activation of Bioenergetic Pathways in Skeletal Muscle**
FRIDAY, JUNE 1, 2012 3:15 PM - 5:15 PM
ROOM: 2001

486 **3:15 PM - 3:20 PM**
Chair: Harry B. Rossiter, FACSM. *University of Leeds, Leeds, United Kingdom.*
(No relationships reported)

487 **3:20 PM - 3:45 PM**
The Dynamics of Skeletal Muscle Bioenergetics
L Bruce Gladden, FACSM. *Auburn University, Auburn, AL.*
(No relationships reported)

488 **3:45 PM - 4:10 PM**
The Sensitisation of Oxidative Metabolism in Whole Muscles and Single Fibers
Harry B. Rossiter, FACSM. *University of Leeds, Leeds, United Kingdom.*
(No relationships reported)

489 **4:10 PM - 4:35 PM**
The Mitochondrial Membrane and Redox Potentials at the Onset of Muscle Contractions
Michael C. Hogan, FACSM. *University of California-San Diego, La Jolla, CA.*
(No relationships reported)

490 **4:35 PM - 5:00 PM**
Exercise-Induced Increases in Mitochondrial Respiratory Sensitivity
P Darrell Neufer. *East Carolina University, Greenville, NC.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-46 Symposium **- Skeletal Muscle Blood Flow Studied Sans Metabolism: Implications from Basic Science to Rehabilitative Medicine**
FRIDAY, JUNE 1, 2012 3:15 PM - 5:15 PM
ROOM: 2014

491 **3:15 PM - 3:20 PM**
Chair: Russell S. Richardson. *University of Utah, Salt Lake City, UT.*
(No relationships reported)

492 **3:20 PM - 3:45 PM**
Impact of Body Position and Afferent Feedback on Central and Peripheral Hemodynamic Contributions to Movement-Induced Hyperemia: Implications for Rehabilitative Medicine
Joel D. Trinity. *University of Utah, Salt Lake City, UT.*
(No relationships reported)

493 **3:45 PM - 4:10 PM**
Attenuated Exercise Induced Hyperaemia with Age: Mechanistic Insight from Passive Limb Movement
John McDaniel. *Kent State University, Cleveland, OH.*
(No relationships reported)

494 **4:10 PM - 4:35 PM**
Passive Limb Movement: A New Tool for Assessing Nitric-Oxide Mediated Vascular Function
Russell S. Richardson. *University of Utah, Salt Lake City, UT.*
(No relationships reported)

495 **4:35 PM - 5:00 PM**
Passive Movement Training: Impact on Angiogenic Factors and Capillary Growth in Human Skeletal Muscle
Ylva Hellsten. *University of Copenhagen, Copenhagen, WY, Denmark.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-47 Tutorial Lecture **- The Relations of Resistance Training and Strength with Morbidity and Mortality**
FRIDAY, JUNE 1, 2012 3:15 PM - 4:05 PM
ROOM: 3014

496 **Allen W. Jackson, FACSM.** *University of North Texas, Denton, TX.*
(No relationships reported)

497 **Jakob L. Vingren.** *University of North Texas, Denton, TX.*
(No relationships reported)

APPENDIX I

Oral Presentation: 2nd International Conference on Hyperphagia in Baton Rouge, LA, October 2012.

HYPERPHAGIA
The Mechanism   The Research   The Treatment

2nd International Conference on Hyperphagia October 17-20, 2012
Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA, USA

Conference Presentations

- Dr. Jack Yanovski: Defining Hyperphagia
  http://youtu.be/chnBReFMEPo
- Dr. Randy Seeley: PPARy in Central Control of Feeding
  http://youtu.be/ptc9DxBQLp0
- Dr. Daniel Driscoll: Prader-Willi Syndrome
  http://youtu.be/KM_IBTDGztQ
- Dr. Nicole Avena: Addictive Behavior and Hyperphagia
  http://youtu.be/iQVlA6aPkBg
- Dr. Anthony Goldstone, Dr. Frank Greenway, Dr. Linda Gourash: Drugs vs. Behavior
  http://youtu.be/GZk5n7BGKGQ
- Dr. Anthony Goldstone, Dr. Christian Vaisse, Dr. Ann Scheimann: Bariatric Surgery
  http://youtu.be/UhGkWB2ttL4
- Conference on Hyperphagia: Discussion
  http://youtu.be/rcMVFQqg_r0
- Conference on Hyperphagia: Panel Facilitated Discussion of Research Challenge Questions
  http://youtu.be/5KtE8XZCCSo
- Dr. George Bray: Etiology and Pathophysiology of Obesity
  http://youtu.be/x_YVpo67I6o

APPENDIX J

CSUF IRB Approval Cover Letter

REAPPROVAL NOTICE

From the Institutional Review Board
California State University Fullerton

Date: December 19, 2012

From: Ron Oliver, Chair

To: **Dr. Daniela Rubin**
Department: Kinesiology, KHS-121

Re: Use of Human Subjects in Research Project entitled:
               Exercise Aspects of Prader Willi Syndrome and Childhood Obesity

The forms you submitted to this office requesting continued approval for the use of human subjects in the above-referenced proposal were reviewed by the California State University Fullerton, Institutional Review Board ("CSUF IRB") at its fully convened meeting held on **December 14, 2012.** Your request for continuation of your research protocol has been approved.

The CSUF IRB has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval notice does not replace any departmental or additional approvals which may be required.

If the above-referenced project has not been completed by **December 13, 2013** you must request renewed approval for continuation of the proposal. The regulations allow for approval on a yearly basis and not to exceed 365 days. This notice is based upon receipt of your request for renewal, and the previous expiration date for your study (not the date on this notice). There is no grace period. If you have not completed your project by the above date, you must stop until renewed approval is secured.

It is of utmost importance that you strictly adhere to the guidelines for human participant and that you follow the plan/methodology/procedures described in your research proposal. Any change in protocol or consent form procedure requires resubmission to the CSUF IRB for approval prior to implementation. Additionally, the principal investigator must promptly report, in writing, any unanticipated or adverse events causing risks to research participants or others.

Please be advised that if you are seeking external funding for this proposal, the above-referenced title should match exactly with the title submitted to the funding sponsor. Any change in project title should be submitted to the CSUF IRB prior to implementation.

By copy of this notice, the chairman of your department (and/or co-investigator) is reminded that s/he is responsible for being informed concerning research projects involving human participants in the department, and should review all protocols of such investigations as often as needed to ensure that the project is being conducted in compliance with our institutional policies and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protections. The Assurance Number is FWA00015384.

Cc: Dr. Dan Judelson
Application No. HSR-12-0478

REAPPROVAL NOTICE
From the Institutional Review Board
California State University Fullerton

Date: December 19, 2012

From: Ron Oliver, Chair

To: **Dr. Daniela Rubin**
Department: Kinesiology, KHS-121

Re: Use of Human Subjects in Research Project entitled:
Resistance exercise aspects of Prader-Willi Syndrome and childhood obesity

The forms you submitted to this office requesting continued approval for the use of human subjects in the above-referenced proposal were reviewed by the California State University Fullerton, Institutional Review Board ("CSUF IRB") at its fully convened meeting held on **December 14, 2012**. Your request for continuation of your research protocol has been approved.

The CSUF IRB has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval notice does not replace any departmental or additional approvals which may be required.

If the above-referenced project has not been completed by **November 12, 2013** you must request renewed approval for continuation of the proposal. The regulations allow for approval on a yearly basis and not to exceed 365 days. This notice is based upon receipt of your request for renewal, and the previous expiration date for your study (not the date on this notice). There is no grace period. If you have not completed your project by the above date, you must stop until renewed approval is secured.

It is of utmost importance that you strictly adhere to the guidelines for human participant and that you follow the plan/methodology/procedures described in your research proposal. Any change in protocol or consent form procedure requires resubmission to the CSUF IRB for approval prior to implementation. Additionally, the principal investigator must promptly report, in writing, any unanticipated or adverse events causing risks to research participants or others.

Please be advised that if you are seeking external funding for this proposal, the above-referenced title should match exactly with the title submitted to the funding sponsor. Any change in project title should be submitted to the CSUF IRB prior to implementation.

By copy of this notice, the chairman of your department (and/or co-investigator) is reminded that s/he is responsible for being informed concerning research projects involving human participants in the department, and should review all protocols of such investigations as often as needed to ensure that the project is being conducted in compliance with our institutional policies and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protections. The Assurance Number is FWA00015384.

Cc: Dr. Dan Judelson
Application No. HSR-12-0477